Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

Nitromethane

EC Number: **200-876-6**

CAS Number: **75-52-5**

Index Number: **609-036-00-7**

Contact details for dossier submitter:

Belgian Federal Public Service Health, Food Chain Safety and Environment
Risk Management service
Galileelaan 5/2
1210 Brussels
Belgium

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SUPPORT ON HOW TO COMPILE ANNEX I TO THE CLH REPORT

Annex I to the CLH report may be compiled from DARs, CARs and/or other sources. Non-confidential DAR/CAR can be annexed as such provided that it has sufficient level of details on the studies. The DS is encouraged to remove any irrelevant parts of the DAR/CAR. The DS must ensure that Annex I can be published during PC, i.e. it does not contain any confidential information.

For support, below is an example on how each study could be presented individually under its own subchapter including the study reference, detailed study summary and results. The format of the detailed study summary of an individual study is flexible as long as the summary is clearly reported and under a correct hazard class. Detailed support can be found below under each subchapter. If DAR/CAR is annexed to the CLH report as Annex I, it must be indicated clearly in the evaluation part of the report where in Annex I the relevant study can be found. If read-across to structurally or mechanistically similar substance is used please provide a justification for using data from this substance and, if known, present the calculations to convert dose/concentration levels from the test substance to the substance for which CLH is proposed. Please provide also a justification for providing non-testing data by any other approaches such as quantitative structure-activity relationships (QSARs) or grouping methods. Support on grouping of substances and read-across can be found in the following links:

http://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf

http://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf

http://echa.europa.eu/documents/10162/13655/pg_report_readacross_en.pdf

http://www.qsartoolbox.org/

http://www.oecd.org/chemicalsafety/risk-assessment/groupingofchemicalschemicalcategoriesandread-across.htm

http://echa.europa.eu/en/view-article/-/journal_content/title/assessing-read-across-how-echa-does-it

1 PHYSICAL HAZARDS

Physical hazards not evaluated in this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 Basic toxicokinetics *in vitro/ex vivo* study (Sakurai *et al.*, 1980)

Study reference:

Sakurai *et al.*, 1980. The interaction of aliphatic nitro compounds with the liver microsomal monooxygenase system, Biochemical Pharmacology, 29, 341-345.

Test type

- No guideline
- GLP compliance not specified
- Reliability 2 (according to the registration dossier, but as the study was not made available to the DS, the assessment could not be conducted in depth)

Test substance

- Nitromethane
- Degree of purity not specified

Detailed study summary and results:

The objective of the study was to assess the *in vitro* liver microsomal metabolism of the test substance. The substrates were added once to the *in vitro* system as 1 M methanolic solutions. Then, the release of formaldehyde and nitrite from nitromethane (CAS: 75-52-5, EC: 200-876-6) by rat liver microsomes in the presence of NADPH and dioxygen was assessed at 2, 4, 6 and 8 minutes. The formation of a cytochrome P450-NO complex was observed during the experiment. Nitromethane was metabolized by the liver microsomes into formaldehyde and nitrate (1:1).

Material and methods

- *Species/strain/Sex:* Sprague-Dawley / rats / male
- *Nb of animals/dose:* unknown
- *Mean body weight:* 120 g
- *Method:* the substances were added once to the *in vitro* system as 1 M methanolic solutions. Then, the release of nitrite and formaldehyde from nitromethane was examined at 2, 4, 6 and 8 minutes.
- *Doses:* 50 mM nitromethane
- *Negative control:* yes

- Rats were pretreated with either phenobarbital (80 mg/kg bw/d, during 3 days) or 3-methylcholanthrene (20 mg/kg bw/d, during 2 days).
- Then the animals were kept on an empty stomach for 24 hours before being killed by decapitation the microsomal fraction was prepared according to Frommer *et al.*, 1970 (U. Frommer, V. Ullrich and Hj. Staudinger, Hoppe-Seyler's 2. Physiof. Chem. 351, 903 in 1970).
- Nitrite determination and spectrometry methods were followed as described in V. Ulhich, G. Herrmann and P. Weber, Biochem. Pharmac. 27, 2301 (1978).
- Substrates used: phenylnitromethane, w-nitrostyrene, nitrocyclohexane, nitromethane and tetranitromethane, together with 2-nitropropane. When formaldehyde was produced from nitromethane, a suspension of the substrate in buffer was used. Formaldehyde was determined according to the method used in J. Cochim and J. Axelrod, J. Pharmac. exp. Ther. 125, 105 (1959).

Results

- Formation of a cytochrome P450-NO complex
- Oxidized rat microsomes catalysed the formation of formaldehyde in a not NADPH-dependent reaction
- Cytochrome P450 can metabolize nitromethane into formaldehyde and nitrate.

2.1.2 Dermal absorption toxicokinetics (Anonymous 15, 1990)

Study reference:

Anonymous 15, 1990

Test type

- No test guideline
- GLP-compliant
- Reliability 2 (according to the registration dossier)

Test substance

- ^{14}C-Nitromethane
- Degree of purity of nitromethane ≥ 99 %
- Impurities unknown
- Batch number unknwon

Detailed study summary and results:

72-h before application, the back skin of two female rhesus monkeys was shaved and a 20 squarred centimetre area was designed as the test site by tattooing. A single dermal dose (300 uL ether/ethanol solution containing 5.5 % 14C nitromethane) was applied on the intact skin by means of a disposable plastic syringe equipped with a feeding needle to guarantee a smooth application. Thereafter, the test site was covered with an occlusive plastic foil patch and taped air tight over the test site. Twelve hours after dose

application the patch was removed and the skin was wiped with soap and acetone swabs to remove remaining test material. The swab and the patch were extracted with acetone and ethanol respectively.
The extracts were assayed for radioactivity. Blood samples (i.e., plasma and erythrocytes separately), urine and feces were collected for 72 hours after dosing and assayed for radioactivity. 72 hours after dose application, the test site and an adjacent 1 cm area were excised. Skin and subcutaneous fat were assayed for radioactivity. The skin samples (treated and untreated) were also examined histologically.
No signs of toxicity was observed in any animal. The body weight did not vary of more than 5 % of the starting weight. Histological examination of the skin did not reveal any signs damage or irritation.
In the 72 hours after application, excretion of the test material was estimated to be 15.39 µg nitromethane (± 0.082% of the total dose), 74.3 % of which was found in the urine. 48 hours after exposure, 90.4 % of the total urine radioactivity was excreted. Also, 25.7 % of the total excreted radioactivity was observed in the feces.
The mean maximum concentration in blood plasma levels was of 37.8 ng nitromethane/mL blood plasma (37.8 ppb) for animal A and 40.3 ng nitromethane/mL blood plasma (40.3 ppb) for animal B after 40 minutes to 2 hours and 20 minutes to 6 hours, for animals A and B, resp.. 72 hours after application, the plasma levels in both monkeys were of 5.3 ppb.
In erythrocytes, nitromethane levels reached a maximum of 44.3 ng/g erythrocytes after 20 minutes in animal A. The maximum concentration was of 40.8 ng/g erythrocytes after 2 hours in animal B. 48 hours after exposure, nitromethane could not be detected in the erythrocytes of both animals.
72 hours after exposure, the skin was excised and samples contained 3.49 µg nitromethane (± 0.018 % of the total dose). No nitromethane was detected in subcutaneous fat. 12 hours after exposure, 2.02 µg nitromethane (± 0.011 % of the total dose) was obtained by wiping the skin with soap/water and acetone swabs. Also, 0.76 µg nitromethane (± 0.004 % of the total dose) was recovered in the occlusive patch. The high loss of nitromethane (18.84 mg, ± 99.88 % of the total dose) was explained by the registrant to be due to the volatility of the test material and thus evaporation (in spite of the patch) from the test site. Plus, the registrant claimed that exhaled radioactivity as nitromethane or volatile metabolites could not be detected under the selected test conditions.
Skin samples revealed that absorption of the test material occurred only in negligible quantity.

Material and methods

- Two female Rhesus monkeys (Macaca fascicularis)
- Dose selected as around 10 times the amount used in metalworking fluids (0.25 – 0.75 %, ± 5 % of active substance)
- Prior to exposure: A 20 squarred centimetre area was shaved 72-h before exposure on the animals back. The area was cleaned with isopropanol 24-h before exposure. Ketamine HCl was used to sedate the monkeys (10 mg/kg IM) then a cathether was installed in their leg for blood collection.
- 300 µL of test material (18.84 mg per animal) was applied on the back skin. Occlusive patch made of plastic wrapping foil was then taped on their back.

- 12-h after exposure, the monkeys were again sedated to remove the patch and swab the area 3 times with 2 % solution of soap/water. A fourth swab of acetone was used as final cleaning. Monkeys were then transfered to metabolism cages.
- 72-h after exposure, the monkeys were sedated to excise the testing area and an adjacent centimetre, subcutaneous fat was removed from skin samples. Fat and skin were weighed and freezed before analysis. Urine, feces and blood samples were collected at different intervals :
  - 0-2, 2-4, 4-6, 6-8, 8-10, 10-12 and then every 12 h for urine
  - 0-4, 4-8, 8-12 and then every 12 h for feces
  - 0.33, 0.66, 1, 2, 3, 4, 6, 8, 10, 12 and then every 12 h for blood.

Results

No signs of toxicity was observed in any animal. The body weight did not vary of more than 5 % of the starting weight. Histological examination of the skin did not reveal any signs damage or irritation.

In the 72 hours after application, mean excretion of the test material was estimated to be 15.39 µg nitromethane (± 0.082 % of the total dose), 74.3 % of which was found in the urine. 48 hours after exposure, 90.3 % of the total urine radioactivity was excreted. Also, 25.7 % of the total excreted radioactivity was observed in the feces.

The mean maximum concentration in blood plasma levels was of 37.8 ng nitromethane/mL blood plasma (37.8 ppb) for animal A and 40.3 ng nitromethane/mL blood plasma (40.3 ppb) for animal B after 40 minutes to 2 hours and 20 minutes to 6 hours, for animals A and B, respectively. 72 hours after application, the plasma levels in both monkeys were of 5.3 ppb.

In erythrocytes, nitromethane levels reached a maximum of 44.3 ng/g erythrocytes after 20 minutes in animal A. The maximum concentration was of 40.8 ng/g erythrocytes after 2 hours in animal B. 48 hours after exposure, nitromethane could not be detected in the erythrocytes of both animals.

72 hours after exposure, the skin was excised and samples contained 3.49 µg nitromethane (± 0.018 % of the total dose). No nitromethane was detected in subcutaneous fat. 12 hours after exposure, 2.02 µg nitromethane (± 0.011 % of the total dose) was obtained by wiping the skin with soap/water and acetone swabs. Also, 0.76 µg nitromethane (± 0.004 % of the total dose) was recovered in the occlusive patch. The high loss of nitromethane (18.84 mg, ± 99.88 % of the total dose) was explained by the registrant to be due to the volatility of the test material and thus evaporation (in spite of the patch) from the test site. Plus, the registrant claimed that exhaled radioactivity as nitromethane or volatile metabolites could not be detected under the selected test conditions.

Skin samples revealed that absorption of the test material occurred only in negligible quantity.

In conclusion, no sign of toxicity was seen in the animals. The mean release of nitromethane in urine was of 11.44 µg (0.062 % of the total dose) during the first 72-h. after 24-h, the maximal excretion rate was reached (2.93 µg, equivalenbt to 0.016 % of the total dose) and after 48-h, 90.4 % of the total urine radioactivity was released.

In feces, excretion rate differed between animals A and B with a total excretion of 5.31 µg (0.027 %) and 2.58 µg (0.013 % of the total dose); a maximal excretion rate after 24h of 2.21 µg (0.012 %) and 0.67 µg (0.004 %) and after 48-h, total feces radioactivity was found to be of 96.2 % and 92.3 %, in animals A and B, respectively.

Plasma levels of nitromethane was maximal between 0.33 and 6 h in animal A and between 0.66 and 2 h in animal B with an average of 40.3 ng/mL and 37.8 ng/mL, respectively. Erythrocytes levels of nitromethane were maximal after 0.33 h with 44.3 ng/g and after 2-h with 40.8 ng/g in monkeys A and B, respectively. No nitromethane was further detected in erythrocytes after 48-h in animal A, whereas it was undetectable in animal B already 4h after exposure.

Nitromethane was found in the skin samples (3.49 µg in average) excised after 72-h, but not in subcutaneous fat. In the 12-h swab, nitromethane average amount was determined to be of 2.02 µg and 0.76 µg was recovered from the patch (in average). The registrant claimed that the loss of test material could be attributed to his volatibility.

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 Acute oral toxicity study in rats (Anonymous 16, 1980)

Study reference:

Anonymous 16, 1980

Detailed study summary and results:

6-week old female and male rats were acclimated for a minimum of 4 days before the experiment. After an overnight fasting, 10 animals/sex/dose were exposed by gavage to 0, 600, 800, 1000, 1400 and 1800 mg/kg bw of nitromethane in a 1 % carboxymethyl cellulose suspension. A daily observation was performed at least during 14 days, body weights were measured before dosing and at days 7 and 14. All animals that died during the study period or that were euthanised on day 14 were necropsied. Major organs and body cavities were observed for gross abnormalities.

Because all animals died a the highest dose, a second experiment was performed with two doses (0 and 1600 mg/kg bw) to refine the LD50 with 10 animals/sex/dose.

An exploratory study was made at first with 5 animals/dose and rats were observed during 7 days. The doses first selected were 500, 1000, 1500 and 2000 mg/kg bw.

Test type

- No guideline
- GLP-compliant

- Reliability 2 (according to the registration dossier, but as the study was not made available to the DS, the assessment could not be conducted in depth).

Test substance

- Nitromethane
- *Degree of purity:* 95.88 %
- *Impurities:* 3.31 % nitroethane, 0.55 % 2-nitropropane

Test animals

- *Species/strain/sex:* Rat / SD / both sexes
- *Nb. of animals per sex per dose:* 10 animals/sex/dose
- *Age and weight at the study initiation:* At least 6-week old, mean weight of 200 and 190 g, for males and females, respectively.

Administration/exposure

- *Mode of administration:* oral (gavage)
- *Duration of test/exposure period:* single exposure
- *Doses/concentration levels:* 0, 600, 800, 1000, 1400, 1600 and 1800 mg/kg bw selected after a probe study with 500, 1000, 1500 and 2000 mg/kg bw. The maximum volume did not exceed 5 mL per dosing.
- *Post exposure observation period:* animals daily observed during a 14-day period
- *Control group and treatment:* yes
- *Vehicle:* 1 % carboxymethyl cellulose suspension
- *Statistical methods:* On the basis of the mortality rate per dose, the Finney method was applied to calculate the LD50 value, the 95 % interval of confidence, slope and strandard error (probit analysis, Cambridge Press, 1979).

Results and reliability

- *LD50 or LC50 value with confidence limits if calculated:* The oral LD50 male/female was 1478 mg/kg bw
- *Nb of deaths at each dose level:* In the exploratory study, 2 and 3 rats died within 7 days after being exposed to 1000 and 1500 mg/kg bw nitromethane, respectively. All rats died within 24 h in the group exposed to 2000 mg/kg bw, furthermore, it was noted that they were shaky and nervous approximatively 3 h after exposure. In the main study, the LD50 was calculated to 1506 (IC95: 1370–1602) and 1449 (IC95: 1261–1560) mg/kg bw in males and females, respectively. The LD50 male/female was determined to be 1478 mg/kg bw. In females exposed to 1400 mg/kg bw, 4 died on day 1 and 1 died on day 3, while in males 3 died on day 1. In animals exposed to 1600 mg/kg bw, 7 died on day 1 and 1 on day 4* in females and 6 in day 1 in males. Finally, in animals exposed to 1800 mg/kg bw, all females and 9 males died on day 1 while the last male died on day 3.

  *This animal was not necropsied

Table 1: Mortality rat

Dose level (mg/kg bw)	0	500	600	800	1000	1400	1500	1600	1800	2000
Exploratory	/	0/5	/	/	2/5	/	3/5	/	/	5/5
Main study (males) part-one	0/10	/	0/10	0/10	0/10	3/10	/	/	10/10	/
Main study (males) part two	0/10	/	/	/	/	/	/	6/10	/	/
Main study (females) part one	0/10	/	0/10	0/10	0/10	5/10	/		10/10	/
Main study part two	0/10	/	/	/	/	/	/	8/10	/	/

Additional information that may be needed to adequately assess data for reliability:

- *Clinical signs:* males exposed to 1800 mg/kg bw were observed as hyperactive 7 hours after dosing, and a bloody discharge was seen from females of the same dose group at 7 hours after dosing as well as convulsions after 13 hours. Tremors after 2 and 4 hours of dosing was objectified in animals exposed to 1600 mg/kg bw.
- *Body weight:* In the first study, mean body weights of surviving males and females tested with any dose up to and including 1000 mg/kg bw were comparable to respective control animals. Furthermore, body weight gains in females exposed to 1400 mg/kg bw were inferior to controls (7.1 vs. 14.6 %) but only during the first 7 days of the experiment. In the second study, performed to refine the LD50, body weight gains were inferior in males and females exposed to 1600 mg/kg bw during the first 7 days of experiment (24.3 and 30.3 % in males and controls, respectively, and 6.2 and 16.4 % in females and controls, respectively) however, their body weight gains were superior from day 7 to 14 (22.8 and 14.6 % in males and controls, respectively, and 7.8 and 4.7 % in females and controls, respectively) in the same dose groups.

Table 2: BWG in animals exposed to 1600 mg/kg bw and in controls in the main study

Dose levels (mg/kg bw)		0	1600
BWG (Males) in %	0-7 d	30.3	24.3
	7-14 d	14.6	22.8
BWG (Females) in %	0-7 d	16.4	6.2
	7-14 d	4.7	7.8

- *Gross pathology:* In the first study, haemorrhage of intestines and/or lungs was seen during necropsy. Otherwise, gross necropsy of all animals were normal, except for 4 which had lung infections. In the second study, haemorrhage of the intestine was observed in all animals which died on day 1. All 6 survivors had normal necropsies.
- *Remarks:* the data presented are extracted from the dissemination website or the IUCLID file due to the low readability of the full study report (PDF file).

3.1.1.2 Acute oral toxicity study in rats (Weatherby, 1955)

Study reference:

Weatherby J.H., 1955, Observation on the Toxicity of Nitromethane, 1955 in AMA Archives of Industrial Health, 11, 102-106.

Detailed study summary and results:

Dogs were exposed by gavage to the test substance emulsified in methylcellulose at doses of 125, 250, 500, 1000 or 1500 mg/kg bw (no control group). Mortality was observed daily and body weights were measured at termination. Major organs (lung, liver, spleen, kidney, stomach and small intestine) were screened microscopically and heart and adrenals were assessed only at 1500 mg/kg bw.

Test type

- Not following test guidelines
- Not GLP-compliant.
- Reliability 2 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* dog / strain not specified / sex not specified
- *Nb. of animals per sex per dose:* groups of 5, 2, 6, 2 and 2 dogs received 125, 250, 500, 1000 or 1500 mg/kg bw, resp.
- *Age and weight at the study initiation:* information not available

Administration/exposure

- *Mode of administration:* oral (gavage)
- *Duration of test/exposure period:* single dose
- *Doses/concentration levels:* 125, 250, 500, 1000 and 1500 mg/kg bw
- *Post exposure observation period:* unknown
- *Control group and treatment:* no control group
- *Vehicle:* 20 % nitromethane in 0.3 % aqueous methylcellulose solution
- *Statistical methods:* unknown

Results and reliability

- *LD50 or LC50 value with confidence limits if calculated:* <125 mg/kg bw
- *Number of deaths at each dose level:* In the group exposed to 125 mg/kg bw, it was reported that one dog was euthanised at 24 h, one at 48 h and one at 72 h for unstated reasons. Their tissues were examined. The two remaining animals survived two months, then observation was terminated considering they appeared to be in good state of health. Dogs exposed to 250 mg/kg bw or more died within 30 h or were killed due to moribund state. No data on body weight is available.

Additional information that may be needed to adequately assess data for reliability:

- *Clinical signs:* In several animals, rectal temperature at termination was approximatively 40 °C.
- *Necropsy findings:* Swollen glomeruli, marked fatty changes and proximal and distal kidney tubules were found in animals exposed to 1500 mg/kg bw. In the same dose group, modifications in the liver as follow: central congestion and occasional edema, cells with large nuclei and lightly stained cytoplasm with many small vacuoles, focal necrosis areas were observed. At 1000 mg/kg bw, occasional midzonal necrosis was seen in the liver 24 h after administration. In animals exposed to 500 mg/kg bw, marked fatty changes and occasional areas of haemorrhage, few chronic inflammatory cells were reported in the liver 32 h after exposure. In the three animals killed at 24 h, 48 h and 72 h after exposure to 125 mg/kg bw, slight fatty changes and few lymphocytes were seen in the liver. Regeneration was evidenced with the presence of mitotic cells in the 48 h animal.
- *Remarks:* The full study report was available to the dossier submitter, but only a poor amount of data was available in the PDF document. The data presented are extracted from the dissemination website or the IUCLID file.

3.1.1.3 Acute oral toxicity study in mice (Weatherby, 1955)

Study reference:

Weatherby J.H., 1955. Observation on the Toxicity of Nitromethane, 1995 in AMA Archives of Industrial Health, 11, 102-106, data published

Detailed study summary and results:

5 male mice per dose were orally exposed by gavage to an aqueous solution containing 5 % nitromethane at doses of 1200, 1500 or 1800 mg/kg bw. Animals were observed for 24 h and the mortality rate was assessed.

Test type

- Not following test guideline
- Not GLP-compliant
- Reliability 2 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* Mouse / strain unknown / male
- *Nb. of animals per sex per dose:* 5 male animals exposed to 1200 and 1800 mg/kg bw and 10 males exposed to 1500 mg/kg bw
- *Age and weight at the study initiation:* not reported

Administration/exposure

- *Mode of administration:* oral (gavage)

- *Duration of test/exposure period:* unknown
- *Doses/concentration levels:* 1200, 1500 and 1800 mg/kg bw
- *Post exposure observation period: /*
- *Control group and treatment:* not specified
- *Vehicle: /*
- *Statistical methods:* Statistical methods: assessment of the LD50 according to the log dose probit method from Miller and Tainter (Miller L.C. and Tainter M.L., Estimation of the ED50 and its Error by Means of Logarithmic Probit Graph Paper, Proc.Soc. Exper. Biol and Med, 57:261, 1944)

Results and reliability

- *LD50 or LC50 value with confidence limits if calculated:* 1440 mg/kg bw (1440 ± 110 mg/kg bw)
- *Number of deaths at each dose level:*

Table 3: Mortality rate

Dose (mg/kg bw)	1200	1500	1800
Dead mice	1/5	6/10	4/5

Additional information that may be needed to adequately assess data for reliability:

- *Time of death:* mostly between 8 and 24 hours
- *Clinical signs:* no information available
- *Necropsy findings:* small focal haemorrhage area on the ears of some animals, several hours after exposure.
- *Remarks:* The full study report was available to the DS, but only a poor amount of data was available in the PDF document. The data presented are extracted from the dissemination website or the IUCLID file.

3.1.1.4 Acute oral toxicity study in rabbits (Machle *et al.*, 1940)

Study reference:

Machle W., Scoot E.W., Treon J., 1940. The Physiological Response of Animals to some Simple MonoNitroparaffins and to certain Derivatives of these Compounds, Journal of Industrial Hygiene and Toxicology, 22:8, 315-332.

Detailed study summary and results:

After 24 h of fasting, undiluted nitromethane was orally administered via gavage to rabbits. The doses were not specifically stated, but at least doses of 750 and 1000 mg/kg bw were defined. After administration, the rabbits were freed and observed for 2-3 hours before returning to their cages. When the animals survived, their weight was measured daily until they had gained back any weight loss, then the measurement was taken once or twice a week.

Test type

- Not following TG
- Not GLP-compliant
- Reliability 2 (according to the registration dossier, however very poor quality of the PDF file of the full study report)

Test substance

- Nitromethane
- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* rabbit / strain not specified / sex not specified
- *Nb. of animals per sex per dose:* not reported
- *Age and weight at the study initiation:* not reported

Administration/exposure

- *Mode of administration:* oral (gavage)
- *Duration of test/exposure period:* single administration
- *Doses/concentration levels:* doses not specifically stated, at least 750 and 1000 mg/kg bw
- *Post exposure observation period*: not specified
- *Control group and treatment:* not specified
- *Vehicle:* no
- *Statistical methods:* not specified

Results and reliability

- *LD50 or LC50 value with confidence limits if calculated:* 750 < LD50 < 1000 mg/kg bw
- *Number of deaths at each dose level:* the lethal dose was reported to be between 750 and 1000 mg/kg bw, no more information is available

Additional information that may be needed to adequately assess data for reliability:

- *Time of death (provide individual animal time if less than 24 hours after dosing):* not reported
- *Clinical signs:* 20 to 40 minutes exposure, progressive weakness and collapse, unsteadiness, incoordination, ataxia and respiration modifications were reported. No significant biochemical changes in the blood reported, nor changes in the color or in methemoglobin formation.

3.1.1.5 Acute oral toxicology study (Anonymous 17, 1960)

Study reference:

Anonymous 17, 1960

Detailed study summary and results:

Rats were orally exposed to nitromethane and then their health status was followed for 14 days. All animals were necropsied for gross pathologic assessment. The LD50 was then evaluated to be 1210 mg/kg bw.

Test type

- Not following test guideline
- Not GLP-compliant
- Reliability 4 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* rat / srain not specified / sex not specified
- *Nb. of animals per sex per dose:* not specified
- *Age and weight at the study initiation:* unknown

Administration/exposure

- *Mode of administration:* oral (gavage)
- *Duration of test/exposure period:* single exposure
- *Doses/concentration levels:* at least 500 and 2000 mg/kg bw
- *Post exposure observation period:* 14 days
- *Control group and treatment:* not specified
- *Vehicle:* not specified
- *Statistical methods:* not specified

Results and reliability

- *LD50 or LC50 value with confidence limits if calculated:* 1210 ± 322 mg/kg bw
- *Number of deaths at each dose level:* the LD0 and the LD100 were determined as 500 and 2000 mg/kg bw, respectively. (no more information available)

Additional information that may be needed to adequately assess data for reliability:

- *Time of death (provide individual animal time if less than 24 hours after dosing):* no information available
- *Clinical signs:* the test substance induced narcotic effects in sublethal doses. The first effect seen was anorexia. Slight to moderate liver effects were found in animals which did not receive the lethal dose. Respiratory failure was seen in animals which died from toxic dose.
- *Necropsy findings:* lung hemorrhages and intestinal effects (gaseous fluid in intestinal tract) were found in animals which were exposed to the lethal dose.

3.1.2 Human data

No human data available

3.1.3 Other data

No other data available

3.2 Acute toxicity - dermal route

Hazard class not evaluated in this CLH dossier

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

3.3.1.1 Acute inhalation toxicity study in rats (Anonymous 10, 1956)

Study reference:

Anonymous 10, 1956

Detailed study summary and results:

10 rats were dosed with 12.75 mg/L nitromethane for one hour. An observation period of 48 h was followed.

Test type

- Similar to guideline design, acceptable restrictions.
- Reliability 2 (according to the registration dossier. However extremely poorly available data, the data presented are extracted from the dissemination website or the IUCLID file.

Test substance

- Nitromethane
- *Degree of purity:* unknown
- *Particle size of dust and mist given as mean mass aerodynamic diameter (MMAD) and geometric standard deviation or give other specifications:* not information available
- *Type or preparation of particles (for studies with aerosols):* no information available

Test animals

- *Species/strain/sex:* rat / strain not specified / sex not specified
- *Nb. of animals per sex per dose:* one group of 10 rats
- *Age and weight at the study initiation:* not specified

Administration/exposure

- *Type of inhalation exposure and test conditions:* whole body exposure to vapours
- *Duration of test/exposure period:* 1 h
- *Doses/concentration levels:* 12.75 mg/L air
- *Analytical verification of test atmosphere concentrations:* yes
- *Post exposure observation period:* 48 h
- *Control group and treatment:* no data
- *Statistical methods:* no data

Results and reliability

- *LD50 or LC50 value with confidence limits if calculated:* LD50 : no data; LCLo: 12.75 mg/L
- *Number of deaths at each dose level:* no death reported after exposure and a 48 h period of observation

Additional information that may be needed to adequately assess data for reliability:

- *Time of death:* no mortality reported
- *Clinical signs:* mild sedation and eye irritation were reported in animals during exposure and recovery.
- *Remarks:* Due to the extremely poorly available data, the dossier submitter downgraded the reliability proposed by the registrant to 3. The data presented are extracted from the dissemination website or the IUCLID file.

3.3.1.2 Acute inhalation toxicity study in rats (Dequidt *et al.*, 1973)

Study reference:

Dequidt J., Vasseur P. and Potencier, 1973. Etude toxicologique expérimentale de quelques nitroparaffines. 4. Etude du nitromethane (English translation by Dr. P.J. Baker Jr, IMC Chemical Group, Inc.), Bull Soc Pharm Lille, 29-35.

Detailed study summary and results:

Rats were exposed to vapour of nitromethane at doses of 500, 2500 or 13000 ppm during 6 hours. When exposed to 500 and 2500 ppm, the exposure was performed daily for 3 weeks (5 d/w) and 4 days, respectively. Rats exposed to 13000 ppm were treated once. The methemoglobin was measured as well as the concentration of nitromethane in the liver, lungs, heart and kidney. Based on the results, nitromethane does not seem to induce methemoglobinemia (< 1 %). Nitromethane was only found in the liver of animals exposed to 13000 ppm.

Test type

- Not following test guidelines
- Not GLP-compliant
- Reliability 2 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* unknown
- *Particle size of dust and mist given as mean mass aerodynamic diameter (MMAD) and geometric standard deviation or give other specifications:* /

Test animals

- *Species/strain/sex:* rat / Wistar / sex not specified

- *Nb. of animals per sex per dose:* 8-10 rats (not specified if per dose or in total)
- *Age and weight at the study initiation:* age unknown, mean body weight of 250 g

Administration/exposure

- *Type of inhalation exposure and test conditions:* whole body exposure, vehicle not specified
- *Duration of test/exposure period:* 6 h, daily exposure for 3 weeks (5 d/7), 4 days or 1 day at 500, 2500 and 13000 ppm, respectively
- *Doses/concentration levels:* 500 ppm (1.25 mg/L), 2500 ppm (6.25 mg/L) and 13000 ppm (32.5 mg/L)
- *Analytical verification of test atmosphere concentrations:* yes
- *Post exposure observation period:* no data
- *Control group and treatment:* no data
- *Statistical methods:* not specified

Results and reliability

- *Number of deaths at each dose level:* in the first experiment, all rats died after 6 h when exposed to 13000 ppm. In the second experiment, the rats died after the 4th day of exposure to 2500 ppm. All animals exposed to the lowest dose survived until euthanasia, after 3 weeks of exposure (5 d/w).
- *LC100:* 13000 ppm

Additional information that may be needed to adequately assess data for reliability:

- *Clinical signs:* in the second experiment, no methemoglobin was detected in the blood. Furthermore, nitromethane was not found in the tissue examination. However, the nitrite ion concentration was concluded to be at a high level. Animals exposed to 500 ppm appeared comparable to control after 3 weeks of exposure. Nitromethane was found in liver (0.27 g/100 g) of animals exposed to 13000 ppm.

Table 4: Effects seen in rats after exposure to nitromethane

Dose (ppm)	Nb of exposures	MetHb (%)	NO2 (µg/g organ)				
			Heart	lungs	kidney	Liver	Spleen
500	15	0 - 0.25	Trace	trace	trace	Trace	Trace
2500	4	0	625	372	408	Trace	800
13000	1	0 - 0.1	554	686	311	Trace	350

- *Remarks:* Due to the extremely poorly available data, the dossier submitter downgraded the reliability proposed by the registrant to 3. The data presented are extracted from the dissemination website or the IUCLID file

3.3.1.3 Acute inhalation toxicity study in rabbit (Machle *et al.*, 1940)

Study reference:

Machle W., Scott E.W., Treon J., 1940. The Physiological Response of Animals to some simple Mononitroparaffins and to certain derivatives of these compounds, Journal of Industrial Hygiene and Toxicology, 22(8), 315-332.

Detailed study summary and results:

Rabbits were exposed to doses of nitromethane, during various amounts of time according to the dose. An observation period of at least 2 months was followed. The animals were then necropsied. Body weights were measured before the experiment and then daily. Blood tests were performed daily prior to the experiment and then weekly, red and white blood cells as well as hemoglobin percentage were counted. Surviving animals were followed for 2 months after exposure. Control rabbits were followed for a 6-month period. The LC50 after a 6h exposure was 5000 ppm (12 mg/L).

Test type

- Not following guidelines
- Not GLP-compliant
- Reliability 2 (according to the registration dossier, however extremely poorly available data)

Test substance

- Nitromethane
- *Degree of purity:* unknown
- *Particle size of dust and mist given as mean mass aerodynamic diameter (MMAD) and geometric standard deviation or give other specifications: /*
- *Type or preparation of particles (for studies with aerosols): /*

Test animals

- *Species/strain/sex:* rabbit / strain not specified / sex not specified
- *Nb. of animals per sex per dose:* 2
- *Age and weight at the study initiation:* not specified

Administration/exposure

- *Type of inhalation exposure and test conditions:* whole body exposure
- *Duration of test/exposure period and doses/concentration levels:* 500 ppm (140 h), 1000 ppm (30 h), 2500 ppm (12 h), 5000 ppm (3 or 6 h), 10000ppm (1, 3 or 6 h), 22500 ppm (1 h), 30000 ppm (0.25, 0.5, 1 or 2 h), 50000 ppm (1 h). For lower exposure levels, it is not stated how much time/d the animals were exposed. The maximal concentration used was 5 %.
- *Analytical verification of test atmosphere concentrations:* yes
- *Post exposure observation period:* 2 months
- *Control group and treatment:* yes
- *Statistical methods:* not reported

Results and reliability

- *Number of deaths at each dose level:* all animals died in the group exposed to 10000 ppm for 6 h, 30000 ppm for 2 h or 50000 ppm for 1 h. Half of the animals per group died after being exposed to 5000 ppm for 6 h. No animal died after being exposed to 10000 ppm for 3 h or 30000 ppm for 1 h.

Table 5: Mortality

Dose (ppm)	5000	10000	10000	30000	30000	50000
Exposure (h)	6	3	6	1	2	1
Mortality	1/2	0/2	2/2	0/2	2/2	2/2

- LC100 (1 h): 50000 ppm; LC100 (2 h): 30000 ppm; LC100 (6 h): 10000 ppm
- LC50 (6 h): 5000 ppm (equivalent to 12 mg/L)
- LC0 (1 h): 30000 ppm; LC0 (3 h): 10000 ppm;

Additional information that may be needed to adequately assess data for reliability:

- *Clinical signs:* irritation, convulsion, jerking or twitching in extremities, slight and transient loss of body weight were reported. Stupor, narcosis and slight general anesthesia were observed when animals were exposed to more than 3% nitromethane in air.
- *Necropsy findings*: general visceral and cerebral congestion, in all animals (but less marked in controls). When exposed to high concentrations, upper respiratory tract irritation and acute pulmonary congestion were observed. Liver damage was evidenced in all animals. Furthermore, liver damage of different severity was seen in all animals. Edema, cloudy swelling or pallor were reported in kidneys or myocardium, among others, and it was considered as changes seen in animals after exposed to lethal dose.
- *Remarks:* Due to the extremely poorly available data, the dossier submitter downgraded the reliability proposed by the registrant to 4. The data presented are extracted from the dissemination website or the IUCLID file.

3.3.1.4 Acute inhalation toxicity study in guinea pigs (Machle *et al.*, 1940)

Study reference:

Machle W., Scott E.W., Treon J., 1940. The Physiological Response of Animals to some simple Mononitroparaffins and to certain derivatives of thesecompounds, Journal of Industrial Hygiene and Toxicology, 22(8), 315-332.

Detailed study summary and results:

Animals were exposed to nitromethane as different doses, for different times of exposure, and then a 2-month observation period was followed. LC50(6 h) was determined to be 5000 ppm (12 mg/L).

Test type

- Not following TG
- Not GLP-compliant

- Reliability 2 (according to the registration dossier. However, extremely poorly available data, the data presented are extracted from the dissemination website or the IUCLID file)
- The use of guinea pig is not usually used for acute inhalation toxicity testing.

Test substance

- Nitromethane
- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* guinea pig / strain not specified / sex not specified
- *Nb. of animals per sex per dose:* 2/group
- *Age and weight at the study initiation:* unknown

Administration/exposure

- *Type of inhalation exposure and test conditions:* whole-body exposure with vapour of nitromethane in air
- *Duration of test/exposure period:* see table below, for low doses, it is not mentioned in the report if the exposure happened daily for small periods of time or as one long exposure.
- *Doses/concentration level:* see table below, the max. conc. Was 5 %.

Table 6: Duration and dose of exposure

Doses (ppm)		500	1000	2500	5000	10000	22500	30000	50000
Duration (h)	Batch 1	140	30	12	3	1	1	0.25	1
	Batch 2	/	48	/	6	3	/	0.5	/
	Batch 3	/	/	/	/	6	/	1	/
	Batch 4	/	/	/	/	/	/	2	/

- *Analytical verification of test atmosphere concentrations:* yes
- *Post exposure observation period:* 2 months
- *Control group and treatment:* yes
- *Statistical methods:* unknown

Results and reliability

- *Number of deaths at each dose level:* all animals exposed to 50000 and 30000 ppm died after 1 h of exposure. All animals died after being exposed to 10000 ppm for 6 h, but none died after 1 h exposure. 1 out of 2 guinea pigs died after 6 h expose of 5000 ppm nitromethane. Finally, no animals died after being exposed to 500 ppm nitromethane for 140 h.

Table 7: Number of dead animals per dose level

Dose (ppm)	**500**	**5000**	**10000**	**10000**	**30000**	**50000**
Exposure (h)	140	6	1	6	1	1

Mortality	0/2	1/2	0/2	2/2	2/2	2/2

- LC100(1 h): 50000 ppm; LC100(1 h): 30000 ppm; LC100(6 h): 10000 ppm
- LC50(6 h): 5000 ppm
- LC0(1 h): 10000 ppm; LC0(140 h): 500 ppm

Additional information that may be needed to adequately assess data for reliability:

- *Clinical signs:* irritation, convulsions, jerking/twitching in extremities, light transient loss of weight were reported. When animals were exposed to more than 3 % nitromethane in air, stupor, narcosis, slight general anesthesia were seen.
- *Necropsy findings:* general visceral and cerebral congestion, in all animals (but less marked in controls). When exposed to high concentrations, upper respiratory irritation and acute pulmonary congestion were observed. Liver damage was evidenced in all animals. Furthermore, liver damage of different severity was seen in all animals. Edema, cloudy swelling or pallor were reported in kidneys or myocardium, among others, and it was considered as changes seen in animals after exposed to lethal dose.
- *Remarks:* Due to the extremely poorly available data, the dossier submitter downgraded the reliability proposed by the registrant to 4. The data presented are extracted from the dissemination website or the IUCLID file.

3.3.1.5 Acute inhalation toxicity study with 1-nitropropane (Anonymous 10, 1956)

Study reference:

Anonymous 10, 1956

Detailed study summary and results:

Test type

- No guideline followed
- GLP: no
- Reliability 2 (according to the registration dossier, however only the summary available, no individual data)
- Read-across performed in the registration dossier

Test substance

- **1-nitropropane**
- *Degree of purity:* 96.12 %

Test animals

- *Species/strain/sex:* rat/Wistar/female
- *Nb. of animals per sex per dose:* 6 female rats/dose

Administration/exposure

- *Type of inhalation exposure and test conditions:* vapour, whole body
- *Duration of test/exposure period:* 1 h
- *Doses/concentration levels:* 8.60, 11.02 and 14.40 mg/L
- *Analytical verification of test atmosphere concentrations:* not reported
- *Post exposure observation period:* 7 d

Results and reliability

- *LD50 or LC50 value with confidence limits if calculated:* 11.02 mg/L
- *Number of deaths at each dose level:* 0, 3 and 5 females died after 1 h of exposure resp. at 8.60, 11.02 and 14.40 mg/L

Additional information that may be needed to adequately assess data for reliability:

- *Time of death (provide individual animal time if less than 24 hours after dosing):* 1 h after exposure
- *Clinical signs:* ataxia in all animals after exposure
- *Necropsy findings, including doses affected, severity and number of animals affected:* not reported
- *Potential target organs (if identified in the report):* not reported
- *Other findings:* not reported

3.3.2 Human data

No human data available

3.3.3 Other data

No other data available

3.4 Skin corrosion/irritation

Hazard class not evaluated in this CLH dossier

3.5 Serious eye damage/eye irritation

Hazard class not evaluated in this CLH dossier

3.6 Respiratory sensitisation

Hazard class not evaluated in this CLH dossier

3.7 Skin sensitisation

Hazard class not evaluated in this CLH dossier

3.8 Germ cell mutagenicity

3.8.1 *In vitro* data

3.8.1.1 *In vitro* data on NITROMETHANE

3.8.1.1.1 *In vitro* gene mutation test in bacteria (Mortelmans *et al.*, 1986)

Study reference:

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E., 1986. Salmonella mutagenicity tests. II. Results from the testing of 270 chemicals. Environ Mutagen, 8(suppl. 7), 1-119.

Detailed study summary and results:

Test type

- OECD TG 471
- Non-GLP
- Deviation: 4 instead of 5 strains
- The pre-incubation protocol was used.
- Reliability 2 (according to the registration dossier)
- *number of replicates:* all tests run in triplicate
- *positive and negative control groups and treatment:*
    - *Negative control:* no
    - *Solvent control:* yes
    - *Positive control:*

Strain	Without metabolic activation	With metabolic activation
TA98	4-nitro-o-phenylenediamine	2-aminoanthracene
TA100	sodium azide	2-aminoanthracene
TA1535	sodium azide	2-aminoanthracene
TA1537	9-aminoacridine	2-aminoanthracene

- *evaluation criteria:*
    - *Positive:* dose-related increase in the number of revertants with respect to the negative control (even if the increase was less than two fold);
    - *Negative:* no increase in the number of mutants;
    - *Questionable:* no clear dose response relationship, no reproducible dose-related relationship, or if the response was of insufficient magnitude to support a positive response.

Test substance

- Nitromethane

- *Degree of purity:* > 99 %

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable:* 4 *S. typh.* strains (TA98, TA100, TA1535 and TA1537)
- *Type and composition of metabolic activation system:*
  - *species and cell type:* S9 fraction prepared from liver of Sprague-Dawley rats or Syrian hamsters induced with Aroclor 1254
  - *quantity:* 10 %
- *Test concentrations:* 100, 333.3, 1000, 3333.3 and 10000 µg/plate. Only in TA100, cytotoxicity was observed at the highest concentration tested (no more information available).
- *Vehicle:* DMSO
- *Statistical methods: /*

Results and discussion

- *Cytotoxic concentrations and genotoxic with and without metabolic activation:* The test compound was tested up to 10 mg/plate and cytotoxicity was only observed in TA100 at the highest concentration tested. No precipitation was present in any of the test conditions. The positive control compounds induced a clear increase in the number of revertants. Overall, no significant increase in the frequency of revertant colonies was observed for any of the bacterial strains with any concentration either in presence or in absence of S9 metabolic fraction. Under the test conditions, the compound is therefore considered as non-mutagenic.

Table 8: Ames test results

Dose level (µg/plate)		0	100	333.3	1000	3333	10000	Positive Control
TA100	-S9	82 ± 2.8	104 ± 2.2	106 ± 10.3	92 ± 4.5	101 ±11.3	127 ± 9.1	461 ± 5.9
	+ 10 % hamster S9	104 ±6.8	113 ± 7.5	111 ± 0.6	101 ± 8.7	105 ± 10.0	120 + 3.2	1720 ± 67.7
	+ 10 % rat S9	101 ± 6.1	109 ± 11.0	89 ± 4.7	94 ± 5.5	101 ± 8.4	99 ± 6.1	577 ± 26.1
TA1535	-S9	23 ± 2.0	19 ± 2.6	19 ± 1.3	21 ± 2.0	20 ± 3.0	23 ± 1.5	458 ± 19.8
	+ 10 % hamster S9	11 ± 1.5	10 ± 2.8	10 ± 1.5	11 ± 3.2	12 ± 1.8	14 ± 3.1	421 ± 16.5
	+ 10 % rat S9	9 ± 1.2	13 ±2.8	13 ± 2.1	9 ± 2.0	10 ± 1.9	14 ± 1.3	392 ± 23.1
TA1537	-S9	8 ± 2.6	7 ± 0.9	7 ± 1.2	8 ± 1.0	9 ± 1.7	7 ± 3.0	431 ± 20.9
	+ 10 % hamster S9	11 ± 0.9	13 ± 2.6	12 ± 3.2	13 ± 2.6	15 ± 2.1	12 ± 1.9	510 ± 10.7
	+ 10 % rat S9	12 ± 2.2	4 ± 1.5	4 ± 1.5	5 ± 0.3	3 ± 0.6	2 ± 0.6	221 ± 31.0

TA98	-S9	28 ± 1.5	37 ± 0.3	34 ± 4.3	31 ± 2.8	25 ± 2.6	30 ± 5.2	777 ± 23.2
	+ 10 % hamster S9	40 ± 1.9	43 ± 6.2	33 ± 5.6	44 ± 1.3	41 ± 0.9	36 ± 5.7	1598 ± 76.2
	+ 10 % rat S9	48 ± 4.3	48 ± 3.6	43 ±2.0	47 ± 4.5	37 ± 3.1	39 ± 1.2	511 ± 35.6

- *Statistical results:* /
- *Remark:* it can be stated that it is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations bacteria have actually been exposed. Furthermore, while the test was run in triplicate, it is specified in Mortelmans *et al.* that only the last experimental results are presented in the article. However, we would like to highlight the fact that data was reported as mean + SEM, which raises questions such as: is it the mean of the triplicates? From which data was this mean calculated? Furthermore, justification should be given for choice of tested dose levels (e.g. dose-finding studies).

3.8.1.1.2 *In vitro* gene mutation test in bacteria (Anonymous 27, 1980)

Study reference:

Anonymous 27, 1980

Detailed study summary and results:

The study was not made available to the DS.

Test type

- Not according to OECD TG 471 as the study was conducted prior to adoption of this guideline
- GLP
- Protocol adapted to volatile compounds by performing exposure in airtight dessicator jars.
- Reliability 2 (according to the registration dossier, however full study report not available to the DS)
- *Nb of replicates:* all tests were run in triplacate
- *Positive and negative control groups and treatment:*
  - *Negative control:* not specified
  - *Solvent control:* yes
  - *Positive control:*

	Without metabolic activation	With metabolic activation
TA98	2-nitrofluorene	2-acetylaminofluorene
TA100	N-methyl-N'-nitro-N-nitrosoguanidine	2-anthramine
TA1535	N-methyl-N'-nitro-N-nitrosoguanidine	2-anthramine
TA1537	Quinacrine mustard-2HCl	8-aminoquinoline
TA1538	2-nitrofluorene	2-acetylaminofluorene

- *Evaluation criteria:*

- *Positive:* the mean number of revertants was at least 3 times higher than the mean of the negative control and a dose-response relationship was observed.
- *'Presumptive':* the mean number of revertants was at least 3 times higher than the mean of the negative control but no dose-response relationship was observed.
- *Equivocal:* the increase in the mean number of revertants was > 2 fold but < 3 fold.

Test substance

- *Nitromethane*
- *Degree of purity:* unknown

Administration/exposure

- *Strain or cell type or cell line:* 5 *S. typh.* strains (TA98, TA100, TA1535, TA1537 and TA1538)
- *Type and composition of metabolic activation system:*
  - *species and cell type:* S9 liver homogenate derived from rats induced with Aroclor 1254 (Litoon Bionetics, Kensington MD)
- *Test concentrations:* A concentration resulting in saturated vapour atmosphere (47465 ppm) caused cytotoxicity in strains TA1535 and TA1537. For this reason, a concentration of 23732 ppm (118.7 mg/L) was tested.
- *Vehicle:* not applicable
- *Statistical methods: /*

Results and discussion

- *Genotoxic effects:* No significant increase was observed in the frequency of revertant colonies at a concentration of 23732 ppm in any of the bacterial strains either in presence or in absence of S9 metabolic fraction. As the highest non-cytotoxic concentration did not cause mutagenicity, no additional concentrations were tested to investigate the concentration-response relationship.

3.8.1.1.3 *In vitro* chromosome aberration study in mammalian cells (NTP, 1997)

Study reference:

National Toxicology Program. 1997. Toxicology and carcinogenesis studies of nitromethane (CAS No. 75-52-5) in F344/N rats and B6C3F1 mice. National Toxicology Program Technical Report Series No. 461. U.S. Department of Health and Human Services (USDHHS), Public Health Service, National Institute of Health (NIH), NIH Publication No. 97, 3377.

Detailed study summary and results:

Nitromethane was unable to induce genotoxic effects on Chinese hamster ovary (CHO) cells via chromosomal aberration mechanisms, both in the presence and in absence of metabolic activation.

Test type

- OECD TG 473
- Non-GLP

- Reliability 2 (according to the registration dossier)
- *Positive and negative control groups and treatment:*
    - *Negative control:* distilled water
    - *Solvent control:* no
    - *Positive control:* mitomycin-C (-S9) and cyclophosphamide (+S9)
- *Nb of metaphases analysed:* Scoring of 200 well-spread metaphases per concentration

Test substance

- Nitromethane
- *Degree of purity:* unknown

Administration/exposure

- *Strain or cell type or cell line:* Chinese Hamster Ovary cells
- *Type and composition of metabolic activation system:*
    - *species and cell type:* S9 fraction prepared from liver of Sprague-Dawley rats induced with Aroclor1254
    - *quantity:* not specified
- *Test concentration:* No cytotoxicity was observed at limit concentration
    - 11.5-hour treatment without S9: 1077, 2316 and 4980 µg/mL
    - 2-hour treatment with S9 followed by 11.5 hours incubation with fresh medium: 1077, 2316 and 4980 µg/mL
- *Vehicle:* distilled water
- *Statistical methods:* Not specified. Analyses were conducted on both the dose response curve and individual dose points.
- *Evaluation criteria:*
    - Chromosomal aberration data were presented as percentages of cells with aberrations;
    - *Positive:* a statistically significant ($p<0.05$) difference for one dose point and a significant trend ($p<0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive.
    - *Equivocal:* positive trend test in the absence of a statistically significant increase at any of the doses

Results and discussion

- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* Nitromethane was negative in the *in vitro* chromosome aberration test in CHO cells both with and without metabolic activation at concentrations as high as the limit concentration of 4980 µg/mL.

Table 9: Chromosomal aberration test results in CHO cells

Compound	Dose level (µg/mL)	N cells	N aberrations	% cells with aberrations
Without metabolic activation				
Nitromethane	1077	200	0	0.0
	2316	200	3	1.5
	4980	200	3	1.5
Distilled water	/	200	6	3.0
Mitomycin-C	0.4	25	10	32.0
With metabolic activation				
Nitromethane	1077	200	5	2.5
	2316	200	2	1.0
	4980	200	6	3.0
Distilled water	/	200	3	1.5
Cyclophosphamide	20	25	51	68.0

- *Remark:* It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations cells have actually been exposed.

3.8.1.1.4 *In vitro* sister chromatid exchange test in mammalian cells (NTP, 1997)

Study reference:

National Toxicology Program. 1997. Toxicology and carcinogenesis studies of nitromethane (CAS No. 75-52-5) in F344/N rats and B6C3F1 mice. National Toxicology Program Technical Report Series No. 461. U.S. Department of Health and Human Services (USDHHS), Public Health Service, National Institute of Health (NIH), NIH Publication No. 97, 3377.

Detailed study summary and results:

Nitromethane was unable to induce genotoxic effects on Chinese hamster ovary cells via sister chromatid exchange mechanisms, both in the presence and in absence of metabolic activation.

Test type

- OECD TG 479
- Non-GLP
- Reliability 2 (according to the registration dossier)
- *Positive and negative control groups and treatment:*
    - *Negative control:* distilled water
    - *Solvent control:* no

- *Positive control:* mitomycin-C (-S9) and cyclophosphamide (+S9)

- *Evaluation criteria:*
  - *Positive:* SCE frequency at least 20 % above the background level, if occurring at any single dose: considered as weak evidence of activity, while if occurring at two or more dises, the positivity of the test was determined.
  - *Equivocal:* statistically significant trend (< 0.005) with no response reaching 20 % above the background

Test substance

- Nitromethane
- *Degree of purity:* unknown

Administration/exposure

- *Strain or cell type or cell line:* Chinese Hamster Ovary cells
- *Type and composition of metabolic activation system:*
  - *species and cell type:* S9 fraction prepared from liver of Sprague-Dawley rats induced with Aroclor1254
  - *quantity:* not specified
- *Test concentrations:* No cytotoxicity was observed at limit concentration
  - 26-hour treatment without S9: 497, 1655 and 4965 µg/mL, then fresh medium was added after removal of the medium containing nitromethane and incubation was prolonged by 2-h
  - 2-hour treatment with S9 followed by removal of the medium containing nitromethane, replacement by fresh medium and incubation was prolonged by 26 hours: 497, 1655 and 4965 µg/mL
- *Vehicle:* distilled water
- *Statistical methods:* Not specified. Analyses were conducted on both the dose response curve and individual dose points.

Results and discussion

- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* Nitromethane did not induce SCE in CHO cells in the *in vitro* assay both with and without metabolic activation at concentrations as high as the limit concentration of 4965 µg/mL.

Table 10: SCE assay results in CHO cells

Dose level (µg/ml)		Nb cells	Nb chromosomes	Nb SCEs	SCE/chromosome	Rel. change of SCE/chrom (%)[a]
Without S9						
Nitromethane	497	50	1049	374	0.35	7.06
	1655	50	1049	394	0.37	12.79

	4965	50	1052	411	0.39	17.32
Distilled water	/	50	1048	349	0.33	/
Mitomycin-C	0.001	50	1050	534	0.50	52.72
	0.004	10	209	186	0.88	167.24
With S9						
Nitromethane	497	50	1050	407	0.38	-4.64
	1655	50	1052	383	0.36	-10.43
	4965	50	1051	381	0.36	-10.881
Distilled water	/	50	1053	428	0.40	/
Cyclophosphamide	0.125	50	1051	647	0.61	51.46
	0.500	10	210	241	1.14	182.35

[a]: SCE/chrom in exposed cells compared to SCE/chrom in control cells

- *Remark:* It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations cells have actually been exposed.

3.8.1.1.5 *In vitro* gene mutation study in bacteria (Anonymous 28, 1975)

Study reference:

Anonymous 28, 1975

Detailed study summary and results:

The ability of nitromethane to induce mutagenic effects was evaluated through an *in vitro* gene mutation assay in bacteria, in the presence and in the absence of metabolic activation. Nitromethane did not show any mutagenic potential in this study.

Test type

- Not according to OECD TG 471 as the study was conducted prior the adoption of this guideline
- Non-GLP
- Both the plate test and the suspension test were performed
- Reliability 2 (according to the registration dossier, however only short data available to the DS and the test material was not soluble under treatment conditions (therefore final concentration unknown) and some reporting deficiencies)
- *Positive and negative control groups and treatment:*
  - *Negative control:* not specified
  - *Solvent control:* yes
  - *Positive control:*

	Without metabolic activation	With metabolic activation

TA1535	Ethylmethane sulfonate	Dimethylnitrosamine
TA1537	Quinacrine mustard	2-Acetylaminofluorene
TA1538	2-Nitrofluorene	2-Acetylaminofluorene
Saccharomyces cerevisiae (D4)	Ethylmethane sulfonate	Dimethylnitrosamine

- *Solubility and stability of the test substance in vehicle if known:* chemical was not soluble under treatment conditions and formed an emulsion

Test substance

- Nitromethane
- *Degree of purity:* unknown

Administration/exposure

- *Strain or cell type or cell line:* 3 *S. typh.* strains (TA1535, TA1537 and TA1538) and 1 *Saccharomyces cerevisiae* (D4)
- *Type and composition of metabolic activation system:*
  - *species and cell type:* whole tissue samples and S9 fraction prepared from lung, liver and testis from mouse (ICR random bred adult males), rat (Sprague-Dawley adult males) and primate (Macaca mulatta adult males) induced with mixture of polychlorinated biphenyls
  - *quantity:* 7.2 mg tissue homogenate or cell fraction/plate
  - *co-factors used:* not clear wether co-factors were used
- *Test concentrations:* Dose-finding study was performed to determine ¼ and ½ of the dose inducing 50 % survival. If no cytotoxicity was obtained for a given strain, a maximum dose of 5 % (w/v) was used. See the selected concentrations below.

	Bacteria	Yeast
¼ 50 % survival	0.25 %	2.5 %
½ 50 % survival	0.50 %	5.0 %
50 % survival	1.00 %	>5.0 %
Plate test	0.50 %	/

- *Vehicle:* buffer
- *Statistical methods*

Results and discussion

- Although not performed according to OECD TG 471, the overall quality of the test appears to be acceptable (dose-range finding, concurrent positive and negative controls, with and without metabolic activation,…). However, some important comments can be made. The compound was not soluble under treatment conditions, and consequently, it is not clear to which concentrations cells have been exposed. Furthermore, no special measures were taken to ensure exposure to volatile compounds. There is also some ambiguity related to the reporting of the results obtained with the suspension test in TA1537. In the summarizing table, a Salmonella reversion frequency of 2.00 x 10^{-}

^{8} is reported as negative control value for the positive control whereas a value of 4.85 x 10^{-8} is used for the test sample. In the detailed reports, the opposite is found. If the numbers have been swapped, this has an impact on the interpretation of the results. Indeed, in this case, the positive control compound does not show a clear positive result whereas the test substance shows a 3-fold increase in reversion frequency at the highest concentration tested. Consequently, data of this study should be interpreted with caution.

- The study was disregarded due to poor data reporting

3.8.1.1.6 *In vitro* gene mutation study in bacteria (Dayal *et al.*, 1989)

Study reference:

Dayal, R., Gescher, A., Harpur, E.S, Pratt, I., and Chipman, K., 1989. Comparison of the Hepatoxicity in Mice and the Mutagenicity of Three Nitroalkanes. Fundamental and Applied Toxicology, 13, 341-348.

Detailed study summary and results:

The ability of nitromethane, nitroethane and 2-nitropropane to induce mutagenic effects was evaluated and compared through an *in vitro* gene mutation assay in bacteria, in the presence and in the absence of metabolic activation. Nitromethane did not show any mutagenic potential in this study.

Test type

- OECD TG 471
- Deviations: only 3 strains tested without metabolic activation
- Non-GLP
- The pre-incubation protocol was used.
- Reliability 2 (according to the registration dossier, but reporting deficiencies (doses not clearly stated for example))
- *Positive and negative control groups and treatment:*
  - *Negative control:* not specified
  - *Solvent control:* yes
  - *Positive control:* not specified
- *Evaluation criteria:*
  - *Positive:* the bacterial count was three times the number of colonies in the solvent controls;

Test substance

- Nitromethane
- *Degree of purity:* unknown

Administration/exposure

- *Strain or cell type or cell line:* 3 *S. typh.* strains (TA98, TA100 and TA102)

- *Type and composition of metabolic activation system:* Not used based on lack of requirement for this enzyme preparation in case of 2-nitropropane mutagenicity (according to Fiala *et al.*, 1987)
- *Test concentrations:* Not clearly specified but up to 200 µmol/plate since nitromethane was toxic to bacteria at a 500 µmol/plate concentration
- *Vehicle:* DMSO
- *Statistical methods:* Mann-Whitney U-test

Results and discussion

- Nitromethane was negative in the *in vitro* gene mutation test but the test was only performed in 3 strains and in absence of S9 metabolic fraction.
- *Remark:* It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations cells have actually been exposed. However, 2-nitropropane induced a positive result in the same study at a low concentration (20 µmol/plate) suggesting that test material remained in solution.

3.8.1.1.7 *In vitro* transformation study in mammalian cells (Kerckaert *et al.*, 1996)

Study reference:

Kerckaert, G.A., Brauninger, R., LeBoeuf, R.A. and Isfort, R.J., 1996. Use of the Syrian Hamster Embryo Cell Transformation Assay for Carcinogenicity Prediction of Chemicals Currently Being Tested by the National Toxicology Program in Rodent Bioassays. Environ Health Perspect, 104(Suppl 5), 1075-1084.

Detailed study summary and results:

Test type

- *In vitro* cell transformation test in Syrian hamster embryo (SHE) cells performed according to EU Method B.21
- Non-GLP
- Reliability 2 (according to the registration dossier)
- *Nb of replicates:* Two individual trials, each consisting of 5 test chemical concentrations, a solvent control and a positive control
- *Criteria for evaluating results:*
    - *Positive:* the compound causes a statistically significant ($p<0.05$) positive dose-response trend test either in the 24 h and/or the 7-day exposure scenario.

Test substance

- Nitromethane
- *Degree of purity:* unknown

Administration/exposure

- *Strain or cell type or cell line:* Syrian hamster embryo (SHE) cells

- *Type and composition of metabolic activation system:* not used
- *Test concentrations:* 0, 2000, 2500, 3000, 3500, 4000 and 5000 µg/mL (= top dose)
    - Exposure for 24 h followed by 6-7 d of growth
    - Exposure for 7 d
- *Vehicle:* DMSO
- *Statistical methods:* Fisher's exact test to compare the transformation frequency of the solvent control pairwise with each test chemical dose group + a trend test on the pooled transformation frequency/dose group data.

Results and discussion

- *Genotoxic effects:* Nitromethane induced a dose-dependent increase in the morphological transformation frequency which was statistically significant compared to the negative control at the two highest concentrations tested.

Table 11: SHE cells transformation test results

Dose level (µg/mL)	0	2000	2500	3000	3500	4000	5000
RPE (%)	100	86	86	92	84	84	76
Nb mutants	5	10	7	8	10	12	14
Nb tot. colonies	1534	1320	1319	1375	1259	1250	949
% mutants/ colonies	0.325	0.75	0.53	0.58	0.79	0.96*	1.47*

RPE= relative plating efficiency (dose group plating efficiency / control group plating efficiency)*100

- *Remark:* An *in vitro* micronucleus test performed in SHE cells was negative. Consequently, the positive result observed in te SHE cell transformation test is probably induced by nonmutagenic mechanisms.

3.8.1.1.8 *In vitro* micronucleus test in SHE cells (Gibson *et al.*, 1997)

Study reference:

Gibson D.P., Brauniger R., Shaffi H.S., Kerckeart G.A., LeBoeuf R.A., Isfort R.J., Aardema M.J., 1997. Induction of micronuclei in Syrian hamster embryo cells: comparison to results in the SHE cells transformation assay for national toxicology program test chemicals, Mutation Research, 392, 61-70.

Detailed study summary and results:

Test type

- *In vitro* micronucleus test in Syrian hamster embryo (SHE) cells
- Non-GLP
- Reliability 2 (according to the registration dossier)
- *Positive and negative control groups:*

- *Negative control:* not specified
- *Solvent control:* DMSO or media
- *Positive control:* colchicine or cyclophosphamide

- *Criteria for evaluating results:* in each dose group, an assessment of the percentage of binucleated cells and of the number of micronucleated cells was performed on 500 cells and 1000 binucleated cells, respectively. Only micronuclei that were non-refractile, completely in the cytoplasm, distinctly separated from the nucleus, and that measured less that 33 % of the nucleus were taken into account.

Test substance

- Nitromethane
- *Degree of purity:* unknown

Administration/exposure

- *Strain or cell type or cell line:* Syrian hamster embryo (SHE) cells
- *Type and composition of metabolic activation system:* not used
- *Test concentrations:* 0 (DMSO), 5.0, 5.5 and 6.0 µg/mL and 0 (media), 3500, 4000, 5000 (µg/mL)
- *Vehicle:* DMSO
- *Statistical methods:* Fisher's exact test

Results and discussion

- *Genotoxic effects:* Nitromethane did not induce micronuclei

Table 12: SHE cells micronucleus test results

Solvent:	DMSO			
Dose level (µg/mL)	0	5.0	5.5	6.5
% MNBC	2.8	2.8	2.4	2.6
Solvent :	Media			
Dose level (µg/mL)	0	3500	4000	5000
% MNBC	0.8	1.3	1.0	0.9

MNBC= micronucleated binucleated cells

- *Remark:* As mentioned above, this *in vitro* micronucleus test performed in SHE cells was negative. Consequently, the positive result observed in the SHE cell transformation test is probably induced by non-mutagenic mechanisms.

3.8.1.1.9 Other studies

Results of an additional *in vitro* study were provided but as the relevance of the study (i.e. induction of DNA damage and/or repair by measuring p53 levels in NCTC 929 cells with ELISA and Western blot analysis, Duerksen-Hughesd *et al.*, 1999) is considered to be limited and results were negative, the study will not be

included in the CLH report. Another study (Gocke *et al.*, 1981) was made available by the registrant but the quality of the report is very limited and assessment is not possible. The study will not be presented in the CLH report.

***In vitro* p53 induction assay (Duerksen-Hughes *et al.*, 1999)**

Study reference:

Duerksen-Hughes P.J., Yang J., Ozcan O., 1999. p53 induction as a genotoxic test for twenty-five chemicals undergoing in vivo carcinogenicity testing, Environ Health Perspect, 107, 805-812.

Detailed study summary and results:

Test type

- No guideline
- Non-GLP
- Reliability 2 (according to the registration dossier)

Test substance

- Nitromethane
- *Purity:* unknown

Administration/exposure

- *Cell type:* cultured mammalian cells (NCTC 929 from mouse fibroblasts)
- *Method:* cells are exposed to nitromethane and, at different time points (6 and 17 h post treatment), cells are lysed to measure the level of p53 by ELISA immuno-sorbent assay and/or Western blot assay. A comparison with untreated cells is then performed.
- *Type and composition of metabolic activation system:* not used
- *Test concentrations:* 1, 10, 20 and 50 µg/mL
- *Vehicle:* DMSO
- *Control:*
    - *Negative control:* not specified
    - *Solvent control:* DMSO or media
    - *Positive control:* N-methyl-N'-nitro-nitrosoguanidine or mitomycin C
- *Statistical methods:* Scheffe S-test
- Tests conducted twice or four times
- *Evaluation criteria:*
    - *Positive:* significant increase ($p<0.001$) in p53 level in the treated group, in comparison with the control, in two or more separate tests and at one or both time points (and maximum 1 out of 3 or 4 experiments showing negative results).

Results and discussion

- No cytotoxicity was reported at any dose level.

- Nitromethane did not induce p53 at any time point or at any dose level.

***In vitro* mutagenicity study (Gocke *et al.*, 1981)**

Study reference:

Gocke E., King M.-T., Eckhardt K;, Wild D., 1981. Mutagenicity of cosmetics ingredients licensed by the European Communities, Mutation research, 90, 91-109.

Not assessed due to the poor quality of the PDF file of the full study report.

3.8.1.2 *In vitro* data on NITROETHANE

3.8.1.2.1 *In vitro* gene mutation test in bacteria (Mortelmans *et al.*, 1986)

Study reference:

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E., 1986. Salmonella mutagenicity tests. II. Results from the testing of 270 chemicals. Environ Mutagen, 8(suppl. 7), 1-119.

Detailed study summary and results:

Test type

- Equivalent OECD TG 471
- Non-GLP
- Deviation: 4 instead of 5 strains
- The pre-incubation protocol was used.
- Reliability 2 (according to the registration dossier)
- *Number of replicates:* All tests were run in triplicate.
- *Criteria for evaluating results:*
    - *Positive:* dose-related increase in the number of revertants with respect to the negative control (even if the increase was less than twofold);
    - *Negative:* no increase in the number of mutants;
    - *Questionable:* no clear dose response relationship, no reproducible dose-related relationship, or if the response was of insufficient magnitude to support a positive response.

Test substance

- Nitroethane
- *Degree of purity:* unknown

Administration/exposure

- *Strain or cell type or cell line:* 4 *S. typh.* strains (TA98, TA100, TA1535 and TA1537)
- *Type and composition of metabolic activation system:*

  - *species and cell type:* S9 fraction prepared from liver of Sprague-Dawley rats or Syrian hamsters induced with Aroclor 1254
  - *quantity:* 10 M
- *Test concentrations:* 100, 333.3, 1000, 3333.3 and 10000 µg/plate.
- *Concurrent negative (solvent/vehicle) and positive control data:*
  - *Negative control:* no
  - *Solvent control:* yes
  - *Positive control:*

S. typh. strain	Without metabolic activation	With metabolic activation
TA98	4-nitro-o-phenylenediamine	2-aminoanthracene
TA100	sodium azide	2-aminoanthracene
TA1535	sodium azide	2-aminoanthracene
TA1537	9-aminoacridine	2-aminoanthracene

- *Vehicle:* DMSO
- *Statistical methods:* not applicable

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* Precipitation was observed in the highest concentration tested in most experiments in all the strains.
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* No significant increase in the frequency of revertant colonies was observed for any of the bacterial strains with any dose (up to 10 mg/plate) either in presence or in absence of S9 metabolic fraction. Under the test conditions, the compound is therefore considered as non-mutagenic.

Table 13: Ames test results

Dose level (µg/plate)		0	100	333.3	1000	3333.3	10000	Positive Control
TA100	-S9	119 ± 2.1	109 ± 8.5	115 ± 1.2	99 ± 5.9	122 ± 3.5	116 ± 11.3	402 ± 44.8
	+ 10 % hamster S9	103 ± 3.8	87 ± 12.2	86 ± 3.7	87 ± 8.5	97 ± 11.5	105 ± 4.8	973 ± 88.4
	+ 10 % rat S9	101 ± 8.7	127 ± 7.3	114 ± 10.3	114 ± 5.5	122 ± 6.9	138 ± 1.8	800 ± 18.5
TA1535	-S9	11 ± 1.2	16 ± 0.7	15 ± 1.0	14 ± 2.4	19 ± 3.2	16 ± 2.7	135 ± 18.0
	+ 10 % hamster S9	8 ± 2.0	7 ± 1.5	6 ± 1.5	4 ± 2.0	9 ± 2.1	7 ± 0.9	325 ± 10.4
	+ 10 % rat S9	5 ± 0.9	10 ± 3.5	7 ± 1.3	15 ± 8.6	8 ± 0.9	8 ± 0.6	277 ± 26.0

TA1537	-S9	5 ± 1.9	10 ± 2.0	8 ± 2.2	8 ± 1.2	8 ± 1.0	8 ± 1.5	131 ± 13.5
	+ 10 % hamster S9	4 ± 0.6	5 ± 0.9	3 ± 0.9	4 ± 0.9	3 ± 0.9	4 ± 1.2	233 ± 3.3
	+ 10 % rat S9	6 ± 1.8	5 ± 1.0	8 ± 1.3	4 ± 1.8	4 ± 1.0	4 ± 0.9	136 ± 5.0
TA98	-S9	43 ± 3.6	31 ± 1.2	34 ± 1.3	32 ± 2.6	32 ± 1.3	38 ± 3.8	543 ± 68.0
	+ 10 % hamster S9	32 ± 4.6	27 ± 1.5	26 ± 5.2	33 ± 7.5	28 ± 6.7	31 ± 7.8	560 ± 10.0
	+ 10 % rat S9	32 ± 3.2	41 ± 6.5	32 ± 6.0	37 ± 4.7	39 ± 5.5	28 ± 4.2	199 ± 20.3

- *Concurrent negative (solvent/vehicle) and positive control data:* In all strains, the positive control compounds induced a clear increase in the number of revertants, both in absence and in presence of S9 metabolic fraction.
- *Remark:* It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations bacteria have been exposed. Furthermore, while the test was run in triplicate, it is specified in Mortelmans *et al.* that only the last experimental results are presented in the article. However, we would like to highlight the fact that data was reported as mean + SEM, which raises questions such as: is it the mean of the triplicates? From which data was this mean calculated?

3.8.1.2.2 *In vitro* gene mutation test in bacteria (Anonymous 29, 1980)

Study reference:

Anonymous 29, 1980

Detailed study summary and results:

Test type

- Not according to OECD TG 471 as the study was conducted prior to adoption of this guideline
- GLP-compliant
- Protocol adapted to volatile compounds by performing exposure in airtight dessicator jars.
- Reliability 2 (according to the registration dossier, however DS no access to raw data to confirm the validity of the study)
- *Number of replicates:* All tests were run in triplicate.
- *Positive and negative control groups and treatment:*
    - *Negative control:* not specified
    - *Solvent control:* yes
    - *Positive control*:

S. typh. strain	Without metabolic activation	With metabolic activation
TA98	2-nitrofluorene	2-acetylaminofluorene
TA100	N-methyl-N'-nitro-N-nitrosoguanidine	2-anthramine
TA1535	N-methyl-N'-nitro-N-nitrosoguanidine	2-anthramine
TA1537	Quinacrine mustard-2HCl	8-aminoquinoline
TA1538	2-nitrofluorene	2-acetylaminofluorene

- *Criteria for evaluating results:*
  - *Positive:* the mean number of revertants was at least 3 times higher than the mean of the negative control and a dose-response relationship was observed.
  - '*Presumptive':* the mean number of revertants was at least 3 times higher than the mean of the negative control but no dose-response relationship was observed.
  - *Equivocal*: the increase in the mean number of revertants was > 2 fold but < 3 fold.

Test substance

- Nitroethane
- *Degree of purity:* unknown

Administration/exposure

- *Strain or cell type or cell line:* 5 *S. typh.* strains (TA98, TA100, TA1535, TA1537 and TA1538)
- *Type and composition of metabolic activation system:*
  - *species and cell type:* S9 liver homogenate derived from rats induced with Aroclor 1254 (Litoon Bionetics, Kensington MD)
- *Test concentrations:* A concentration resulting in saturated vapour atmosphere (55450 ppm) caused cytotoxicity in strains TA1535 and TA1537. For this reason, a concentration of 27725 ppm was tested. (no more information available on cytotoxicity)
- *Vehicle:* not applicable
- *Statistical methods:* not applicable

Results and discussion

- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* No significant increase was observed in the frequency of revertant colonies at a concentration of 27725 ppm in any of the bacterial strains either in presence or in absence of S9 metabolic fraction. As the highest non-cytotoxic concentration did not cause mutagenicity, no additional concentrations were tested to investigate the concentration-response relationship.
- *Remark:* the study was not made available to the DS

3.8.1.2.3 *In vitro* gene mutation test in mammalian cells (Anonymous 30, 2012)

Study reference:

Anonymous 30, 2012

Detailed study summary and results: nitroethane's mutagenic potential was evaluated through a CHO/HGPRT gene mutation assay in mammalian cells, in the presence and in the absence of S9. Doses selected were 0, 2.9, 5.9, 11.7, 23.5, 46.9, 93.9, 187.8, 375.5 and 751 µg/mL (highest concentration based on the guideline limit of 10 mM for this test system) and they were used in a preliminary test before being adapted for the initial and confirmatory mutagenic assays. There was no significant increase in the frequency of mutants due to nitroethane, in comparison with the negative control group, in the presence and in absence of S9.

Test type

- OECD TG 476
- GLP-compliant
- Special modifications were made for volatile compounds: more specifically, caps of flasks used in the test were tightly sealed to ensure all test material remained in the flask.
- Reliability 1 (according to the registration dossier)
- *Number of replicates:* All tests were run in duplicate.
- *Positive and negative control groups and treatment:*
    - *Negative control:* distilled water
    - *Positive control:*
        - -S9: ethylmethanesulfonate (621 µg/mL)
        - +S9: 20-methylcholanthrene (4 and 8 µg/mL)
- *Criteria for evaluating results:*
    - *Acceptable test:* the mutant frequency in positive controls was significantly higher than the solvent controls and the mutant frequency in the solvent controls was within reasonable limits of the laboratory historical control values and literature values.
    - *Positive:* the compound induced a statistically significant, dose-related, reproducible increase in mutant frequency.

Test substance

- Nitroethane
- *Degree of purity:* 99.9 %

Administration/exposure

- *Strain or cell type or cell line:* Chinese Hamster Ovary cells (CHO-K1-BH4)
- *Target gene:* HGPRT
- *Type and composition of metabolic activation system:*
    - *species and cell type:* S9 liver homogenate derived from Sprague-Dawley rats induced with Aroclor 1254 (Litoon Bionetics, Kensington MD)
    - *quantity:* 2 %
- *Test concentrations:*

- *Assay 1 (preliminary):* 0, 2.9, 5.9, 11.7, 23.5, 46.9, 93.9, 187.8, 375.5 and 751 µg/mL (= 10mM = limit dose) ± S9
- *Assay 2 (initial mutagenic test):* 0, 46.9, 93.9, 187.8, 375.5, and 751 µg/mL ± S9
- *Assay 3 (confirmatory mutagenic test):* 0, 46.9, 93.9, 187.8, 375.5, and 751 µg/mL ± S9

- *Vehicle:* distilled water
- *Statistical methods:* The frequency of mutants per 10^6 clonable cells was statistically evaluated using a weighted analysis of variance. If the analysis of variance was significant at alpha = 0.05, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control (alpha = 0.05, one-sided). Linear dose-related trend tests were performed if any of the pairwise comparisons of test material with the negative control yielded significant differences.

Results and discussion

- *Preliminary test:* no to low toxicity was observed in the treated cells cultures ± S9 with the relative cell survival (RCS) ranging from 95.7 to 116.8 % in the absence of S9 and 85.5 to 108.2 % in the presence of S9. Concentrations were adapted to of 0, 46.9, 93.9, 187.8, 375.5, and 751 µg/mL of nitroethane for the initial and confirmatory gene mutation assays ±S9.

Table 14: CHO cells survival (number of colonies/plate) after nitroethane exposure in the preliminary test

Dose (µg/mL)			0	2.9	5.9	11.7	23.5	46.9	93.9	187.8	375.5	751
-S9	Test	1	149	174	140	166	169	158	179	160	156	153
		2	139	170	157	173	152	153	172	163	117	172
		3	153	138	164	176	153	170	164	168	149	177
	Avg. RCS (%)		100	109.3	104.5	116.8	107.5	109.1	116.8	111.3	95.7	113.8
+S9	Test	1	148	148	124	138	120	128	131	141	116	130
		2	143	140	113	143	104	121	168	143	117	146
		3	123	138	133	131	130	122	140	164	123	124
	Avg. RCS (%)		100	102.9	89.4	99.5	85.5	89.6	106	108.2	86	96.6

RCS = relative cell survival, [(mean number of colonie/plate) in the treated group/(mean number of colonie/plate) in the controlgroup]*100

- *Initial mutagenic test:* no to moderate toxicity was observed with RCS ranging from 63.3 to 105.5 % in the absence of S9. Minimal toxicity was observed in the presence of S9 with RCS ranging from 91.3 to 109.8 %. The mutant frequencies observed in cultures treated with nitroethane ±S9 at all concentration levels were not significantly changed from the control values.

Table 15: Toxicity results of the initial mutagenic assay (without and with S9)

Dose level (µg/mL)	Assay	**-S9**				**+S9**			
		1	2	3	RCS (%)	1	2	3	RCS (%)
0	1	185	153	173	107.8	147	167	149	104.6

	2	136	152	149	92.2	148	133	141	95.4
46.9	1	166	157	162	102.3	135	163	136	98.1
	2	157	143	145	93.9	147	138	144	96.9
93.9	1	167	167	155	103.2	140	143	121	91.3
	2	165	153	168	102.5	128	153	140	95.1
187.8	1	186	160	154	105.5	167	160	147	107.1
	2	175	142	144	97.3	132	175	156	104.6
375.5	1	120	122	99	71.9	155	173	158	109.8
	2	88	115	97	63.3	156	144	142	99.9
751	1	157	138	150	93.9	152	150	129	97.4
	2	125	158	146	90.5	171	126	148	100.6
Positive control A	1	78	75	82	49.6	160	165	169	111.6
	2	102	79	87	56.5	172	162	163	112.3
Positive control B	1	/	/	/	/	158	161	136	102.8
	2	/	/	/	/	139	138	158	98.3

With S9: positive control A (4 µg/mL) and B (8 µg/mL) of 20-MCA

Table 16: Mutation assay results (without S9), results in duplicate, in the initial test

Dose (µg/mL)	Assay	**Mutation result**	**Cloning efficiency (CE)**				Mutants per million clonable cells
		Total mutant colonies/plate	Test 1	Test 2	Test 3	CE (%)	
0	1	1	166	150	162	79.7	0.6
	2	7	154	138	127	69.8	5.0
46.9	1	20	107	108	124	56.5	17.7
	2	11	146	138	131	69.2	8.0
93.9	1	18	119	117	133	61.5	14.6
	2	11	101	120	128	58.2	9.5
187.8	1	30	104	108	112	54.0	27.8
	2	15	124	119	111	59.0	12.7
375.5	1	9	139	123	134	66.0	6.8
	2	13	144	116	160	70.0	9.3
751	1	8	97	117	103	52.8	7.6
	2	6	136	132	103	61.8	4.9
Positive control	1	210	69	61	82	35.3	297.2*

	2	235	62	82	91	39.2	300.0*

Table 17: Mutation assay results (with S9), in the initial test

Dose (µg/mL)	Assay	**Mutation result**	**Cloning efficiency (CE)**				Mutants per million clonable cells
		Total mutant colonies/plate	Test 1	Test 2	Test 3	CE (%)	
0	1	13	130	127	144	66.8	9.7
	2	20	136	146	143	70.8	15.7
46.9	1	9	106	117	126	58.2	7.7
	2	20	136	139	154	71.5	14.0
93.9	1	8	114	131	112	59.5	7.5
	2	16	101	151	114	61.0	13.1
187.8	1	11	101	105	93	49.8	11.0
	2	29	116	115	128	59.8	24.2
375.5	1	11	73	88	91	42.0	13.1
	2	22	135	106	128	61.5	17.9
751	1	15	130	119	112	60.2	13.9
	2	12	111	108	114	55.5	10.8
Positive control A	1	275	113	102	92	51.2	268.7*
	2	286	106	118	117	56.8	251.6*
Positive control B	1	455	132	104	111	57.8	393.4*
	2	394	98	127	129	59.0	333.9*

With S9: positive control A (4 µg/mL) and B (8 µg/mL) of 20-MCA.

- *Confirmatory test:* no to low toxicity was reported, as indicated by RCS, in the absence of S9 activation (87.4 to 109.8 %). In the presence of S9, RCS showed minimal to no toxicity with values ranging from 79.2 to 97.7 %. The frequency of mutants seen in cell cultures treated with nitroethane ±S9 were not significantly different from the control values, and were within the range of the HCD.

Table 18: Cytotoxicity results in the confirmatory test (with and without S9)

Dose (µg/mL)	Assay	**Mutation result**	**Cloning efficiency (CE)**				Mutants per million clonable cells
		Total mutant colonies/plate	Test 1	Test 2	Test 3	CE (%)	
0	1	2	176	168	178	87.0	1.3
	2	4	192	207	203	100.3	2.5
46.9	1	6	191	210	217	103.0	3.6
	2	2	160	184	170	85.7	1.3

93.9	1	19	214	208	229	108.5	8.8
	2	20	208	196	187	98.5	11.3
187.8	1	9	230	221	199	108.3	4.2
	2	6	257	215	246	119.7	2.8
375.5	1	9	193	195	-	97.0	5.2
	2	4	152	186	197	89.2	2.8
751	1	10	202	188	190	96.7	5.2
	2	19	187	183	170	90.0	11.7
Positive control	1	132	81	84	82	41.2	160.3
	2	160	94	93	104	48.5	164.9

Table 19: Mutation assay results (without S9), results in duplicate, in the confirmatory test

Dose (µg/mL)	Assay	**Mutation result**	**Cloning efficiency (CE)**				Mutants per million clonable cells
		Total mutant colonies/plate	Test 1	Test 2	Test 3	CE (%)	
0	1	13	209	198	205	102.0	6.4
	2	18	243	230	225	116.3	7.7
46.9	1	6	237	238	222	116.2	2.6
	2	16	209	214	228	108.5	7.4
93.9	1	11	208	209	207	104.0	6.6
	2	7	230	205	213	108.0	3.6
187.8	1	10	211	209	209	104.8	4.8
	2	4	162	205	179	91.0	2.2
375.5	1	4	195	196	209	100.0	2.0
	2	8	196	200	180	96.0	4.2
751	1	16	217	209	203	104.8	7.6
	2	10	205	193	191	98.2	5.1
Pos. control A	1	206	160	145	136	73.5	140.1*
	2	277	202	193	195	98.3	140.9*
Pos. control B	1	287	169	173	165	84.5	169.8*
	2	299	162	141	131	72.3	206.7*

*P < 0.05

Table 20: Mutation assay results (with S9) in the confirmatory test

Dose (µg/mL)	Assay	**Mutation result**	**Cloning efficiency (CE)**				Mutants per million clonable cells
		Total mutant colonies/plate	Test 1	Test 2	Test 3	CE (%)	
0	1	13	209	198	205	102.0	6.4
	2	18	243	230	225	116.3	7.7
46.9	1	6	237	238	222	116.2	2.6
	2	16	209	214	228	108.5	7.4
93.9	1	11	208	209	207	104.0	6.6
	2	7	230	205	213	108.0	3.6
187.8	1	10	211	209	209	104.8	4.8
	2	4	162	205	179	91.0	2.2
375.5	1	4	195	196	209	100.0	2.0
	2	8	196	200	180	96.0	4.2
751	1	16	217	209	203	104.8	7.6
	2	10	205	193	191	98.2	5.1
Positive control A	1	206	160	145	136	73.5	140.1*
	2	277	202	193	195	98.3	140.9*
Positive control B	1	287	169	173	165	84.5	169.8*
	2	299	162	141	131	72.3	206.7*

With S9: positive control A (4 µg/mL) and B (8 µg/mL) of 20-MCA.

Table 21: Historical control data for mutant frequency in CHO cells (2007-2012)

Year	S9	Number	Range
2007	-	32	0.7 - 14.5
	+	32	1.3 - 32.2
2008	-	16	2.2 - 26.0
	+	15	2.3 - 24.2
2009	-	12	2.9 - 15.1
	+	12	3.4 - 15.6
2010	-	44	1.6 - 15.2
	+	46	1.6 - 14.3
2011	-	8	1.5 - 11.8

	+	8	0.0 10.3
2012	-	4	4.2 - 11.0
	+	4	5.8 - 9.1

- Nitroethane was non-mutagenic both in absence and in presence of S9 metabolic fraction in the mammalian gene mutation test at concentrations up to the limit concentration.

3.8.1.2.4 *In vitro* gene mutation study in bacteria (Dayal *et al.*, 1989)

Study reference:

Dayal, R., Gescher, A., Harpur, E.S, Pratt, I., and Chipman, K., 1989. Comparison of the Hepatoxicity in Mice and the Mutagenicity of Three Nitroalkanes. Fundamental and Applied Toxicology, 13, 341-348.

Detailed study summary and results:

Test type

- OECD TG 471
- Non-GLP
- Deviation: Only 3 strains tested without metabolic activation
- The pre-incubation protocol was used.
- Reliability 2 (according to the registration dossier, but reporting deficiencies (doses not clearly stated for example))
- *Positive and negative control groups and treatment:*
  - *Negative control:* not specified
  - *Solvent control:* yes
  - Positive control: not specified
- *Criteria for evaluating results:*
  - *Positive:* the bacterial count was three times the number of colonies in the solvent controls.

Test substance

- Nitroethane
- *Degree of purity:* unknown

Administration/exposure

- *Strain or cell type or cell line:* 3 *Salmonella typhimurium* strains (TA98, TA100 and TA102)
- *Type and composition of metabolic activation system:* Not used based on lack of requirement for this enzyme preparation in case of 2-nitropropane mutagenicity (according to Fiala *et al.*, 1987)
- *Test concentrations:* Not clearly specified but not up to 200 µmol/plate since nitromethane was toxic to bacteria at a 500 µmol/plate concentration
- *Vehicle:* DMSO + phosphate buffer (0.2 M, pH 7.4)
- *Statistical methods:* Mann-Whitney U-test

Results and discussion

- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* Nitroethane was negative in the *in vitro* gene mutation test but the test was only performed in 3 bacterial strains and in absence of S9 metabolic fraction.
- *Remark:* It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations cells have actually been exposed. However, 2-nitropropane induced a positive result in the same study at a low concentration (20 µmol/plate) suggesting that the test material remained in solution.

3.8.1.3 *In vitro* data on NITROPROPANE

3.8.1.3.1 *In vitro* gene mutation test in bacteria (Anonymous 31, 1996)

Study reference:

Anonymous 31, 1996

Detailed study summary and results:

Test type

- OECD TG 471
- GLP
- Protocol adapted to volatile compounds: agar plates were placed in individual, sealed stainless steel containers immediately after treatment.
- Reliability 1 (according to the registration dossier)
- *Number of replicates:* Each condition was tested in triplicate.
- *Positive and negative control groups and treatment:*
  - *Negative control :* yes (vehicle control – DMSO)
  - *Positive control :* with met. act. : 2-aminoanthracene (2AA) for all strains
    Without met. act. : 4-nitroquinoline-1-oxide (4NQQ) for TA98, N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) for TA100, TA1535 and WP2uvrA-, 9-aminoacridine (9AA) for TA1537
- *Criteria for evaluating results:*
  - *Positive :* the compound induced at least a 2-fold, dose-dependent increase in mutation rate (with respect to the spontaneous rate) in one or more strains.

Test substance

- 1-nitropropane
- *Degree of purity:* ±99 %
- *Impurities:* other nitroparaffins
- Stable

- Miscible with DMSO

Administration/exposure

- *Strain or cell type or cell line: S. typh.* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA-
- *Type and composition of metabolic activation system:*
  - *species and cell type:* S9 liver homogenate derived from SD rats induced with Aroclor 1254 (prepared in-house)
- *Test concentrations:* 50, 150, 500, 1500 and 5000 µg/plate (= limit dose). No cytotoxicity was observed in any of the strains at the highest concentration tested.
- *Vehicle:* DMSO

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* no (no more information available)
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* negative
- *Mean number of revertant colonies:*

Table 22: Mean number of revertant colonies

Strain	Dose (µg/plate)	Mean number of revertants/plate			
		Without met. act.		With met. act.	
		Trial 1	Trial 2	Trial 1	Trial 2
TA100	0	116 ± 13.3	106 ± 7.9	127 ± 2.5	93 ± 7.6
	50	117 ± 3.2	100 ± 20.5	123 ± 10.4	101 ± 9.3
	150	102 ± 5.0	106 ± 13.0	126 ± 4.0	95 ± 8.9
	500	115 ± 8.3	95 ± 4.6	129 ± 5.3	97 ± 10.7
	1500	121 ± 10.1	106 ± 8.1	115 ± 3.0	126 ± 47.0
	5000	111 ± 9.1	98 ± 8.0	121 ± 1.7	95 ± 4.9
	Positive control	865 ± 18.5	514 ± 59.7	1203 ± 162.4	1389 ± 31.2
TA1535	0	16 ± 4.0	21 ± 4.0	18 ± 3.1	17 ± 2.5
	50	16 ± 2.0	21 ± 3.2	17 ± 2.3	16 ± 3.6
	150	15 ± 1.0	24 ± 5.5	16 ± 6.1	13 ± 4.4
	500	16 ± 1.0	26 ± 3.5	13 ± 2.6	14 ± 1.5
	1500	19 ± 3.1	24 ± 1.5	17 ± 3.8	18 ± 2.3
	5000	20 ± 1.0	22 ± 2.9	16 ± 2.1	17 ± 0.6
	Positive control	650 ± 16.6	189 ± 12.3	302 ± 20.2	227 ± 14.0
TA98	0	28 ± 3.2	22 ± 0.6	31 ± 4.0	30 ± 3.6
	50	26 ± 2.5	24 ± 0.6	28 ± 3.1	26 ± 4.4
	150	26 ± 4.2	25 ± 2.6	25 ± 3.5	28 ± 3.1

	500	25 ± 3.6	24 ± 3.1	29 ± 4.6	30 ± 2.5
	1500	25 ± 4.5	21 ± 2.9	28 ± 2.1	22 ± 2.6
	5000	26 ± 1.5	19 ± 2.1	29 ± 2.0	27 ± 9.7
	Positive control	254 ± 7.0	168 ± 13.8	582 ± 58.4	602 ± 65;5
TA1537	0	11 ± 2.3	12 ± 1.5	9 ± 0.0	12 ± 1.0
	50	8 ± 1.5	14 ± 1.2	8 ± 2.6	10 ± 2.0
	150	9 ± 0.6	10 ± 1.5	12 ± 1.7	13 ± 3.1
	500	9 ± 2.1	10 ± 2.1	12 ± 3.5	14 ± 2.5
	1500	8 ± 1.5	10 ± 1.0	12 ± 3.1	13 ± 1.2
	5000	9 ± 2.5	11 ± 4.6	9 ± 1.0	11 ± 1.5
	Positive control	986 ± 70.8	794 ± 106.0	404 ± 31.5	412 ± 35.3
WP2uvrA-	0	28 ± 3.2	22 ± 4.2	28 ± 2.1	22 ± 3.1
	50	28 ± 9.1	19 ± 1.5	25 ± 1.5	25 ± 3.5
	150	28 ± 5.5	23 ± 5.7	25 ± 2.1	19 ± 2.9
	500	24 ± 1.7	18 ± 3.1	30 ± 1.5	23 ± 3.1
	1500	31 ± 2.0	24 ± 4.7	27 ± 2.5	23 ± 5.7
	5000	31 ± 2.6	23 ± 3.1	26 ± 5.3	22 ± 3.2
	Positive control	1035 ± 26.6	705 ± 22.1	959 ± 43.5	730 ± 35.1

3.8.1.3.2 *In vitro* chromosome aberration study in mammalian cells (Anonymous 32, 1994)

Study reference:

Anonymous 32, 1994

Detailed study summary and results:

Test type

- No guideline followed
- GLP
- Reliability 2 (according to the registration dossier)
- *Number of replicates:* Each concentration was tested in duplicate.
- *Positive and negative control groups and treatment:*
    - *Negative control:* yes (vehicle control)
    - *Positive control:* mitomycin-C (for 24 and 48 h treatment) and cyclophosphamide (for 6 h treatment with and without met. act.)
- *Number of metaphases analysed:* Scoring of 100 well-spread metaphases per concentration (when possible) – 200 metaphases per concentration
- Precipitation of 1-nitropropane observed at and above 2500 µg/mL
- *Criteria for evaluating results:*

- *Positive result:* no criteria specified.

Test substance

- 1-nitropropane
- *Degree of purity:* >99 %

Administration/exposure

- *Strain or cell type or cell line:* Chinese Hamster Lung (CHL) cells
- *Type and composition of metabolic activation system:*
  - *species and cell type:* S9 fraction prepared from liver of SD rats induced with Aroclor1254
  - *quantity:* 5 %
- *Test concentrations:* A preliminary cytotoxicity test was performed and growth inhibition was estimated by counting the number of cells at the end of the culture period. In all cases, 1-nitropropane showed evidence for cell toxicity and more toxicity was observed after the 48 hour treatment. Based on these results, the following concentrations were selected :
  - 6-hour treatment without S9 : 625, 1250, 2500 and 5000 µg/mL
  - 24- and 48-hour treatment without S9 : 312.5, 625, 1250 and 5000 µg/mL
  - 6-hour treatment with S9 : 156.25, 312.5, 625, 1250, 2500 and 5000 µg/mL
  - Comment : 3 concentrations were scored for chromosome aberrations.
- *Vehicle:* DMSO

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* yes,

  Viability decreased with increasing incubation time.

Table 23: Percentage of cell viability

Without met. act.						With met. act.	
24 h treatment		48 h treatment		6 h treatment		6 h treatment	
Conc.	% of cell viability	Conc.	% of cell viability	Conc.	% of cell viability	Conc.	% of cell viability
NC	100	NC	100	NC	100	NC	100
312.5	100	312.5	72	625	93	625	99
625	85	625	57	1250	87	1250	102
1250	80	1250	52	2500	75	2500	93
2500	62	2500	32	5000	15	5000	74
MMC	67	MMC	81	CP	106	CP	77

Conc. : in µg/mL ; NC : negative control ; MMC : mitomycine C ; CP : cyclophosphamide

- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* negative, no concentration-related increase in the frequency of cells with chromosome aberrations either in the presence or absence of a metabolic activation at any of the exposure times.

Table 24: Total number of cells with chromosome aberration

Without met. act.						With met. act.	
24 h treatment		48 h treatment		6 h treatment		6 h treatment	
Conc.	Cells with abs	Conc.	Cells with abs	Conc.	Cells with abs	Conc.	Cells with abs
NC	4/200	NC	3/200	NC	2/200	NC	2/200
312.5	NE	312.5	4/200	625	4/200	625	NE
625	5/200	625	8/200	1250	10*/200	1250	2/200
1250	7/200	1250	8/200	2500	5/200	2500	0/200
2500	7/200	2500	toxic	5000	Toxic	5000	3/200
MMC	65***/150	MMC	97***/100	CP	4/200	CP	78***/100

Conc. : in µg/mL ; *** : p<0.001 ; NE : not evaluated ; NC : negative control ; MMC : mitomycin-C ; CP : cyclophosphamide

- *Remark:* Results obtained after a 6 h treatment period in absence of S9 should not be considered as cyclophosphamide was used as a positive control. Cyclophosphamide did not induce an increase in chromosome aberrations which is not surprising as the compound requires metabolic activation. It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations cells have actually been exposed.

3.8.1.3.3 *In vitro* DNA damage and/or repair study (Andrae U. *et al.*, 1988)

Study reference:

Andrae U., Homfeldt H., Vogl L., Lichtmannegger J. and Summer K.H., 1988. 2-Nitropropane induces DNA repair synthesis in rat hepatocytes in vitro and in vivo, Carcinogenesis, 9(5), 811-815.

Detailed study summary and results:

Test type

- Similar to OECD TG 482 (Following the OECD Council decision, the Test Guideline 482 'Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells *in vitro*' was deleted on 2nd April 2014)
- Non-GLP
- Reliability 2 (according to the registration dossier, however only summary available)
- The protocol for the *in vitro* unscheduled DNA synthesis assay in mammalian cells was adapted for volatile compounds : flasks were closed air tight.
- *Criteria for evaluating results:* no additional information available

Test substance

- 1-nitropropane
- *Degree of purity:* 97.4 %

Administration/exposure

- *Strain or cell type or cell line:* primary hepatocytes obtained from male and female Wistar rats
- *Test concentrations:* 0.1-10 mM – Viability of cells was assessed with trypan blue staining.
- *Vehicle:* /

- *Statistical methods:* not specified

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* no information available
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* negative
- 1-Nitropropane induced an up to 5-fold increase in repair incorporation in hepatocytes from male and female rats. However, the authors reported that this repair induction was attributed to 2-nitropropane that was present as an impurity (2.3 %).
- *Remark:* No access to manuscript or raw data. Results of *in vitro* UDS tests should be interpreted with caution as this test method is considered obsolete and has been deleted from the OECD TG program.

3.8.1.3.4 *In vitro* gene mutation test in mammalian cells (Roscher E. *et al.*, 1990)

Study reference:

Roscher E., Ziegler-Skylakakis K. and Andrae U., 1990. Involvement of Different Pathways in the Genotoxicity of Nitropropanes in Cultured Mammalian Cells, Mutagenesis, 5, 375-380.

Detailed study summary and results:

Test type

- Similar to OECD TG 476 (according to registration dossier, however only summary available, not enough information to confirm the validity of the study)
- Non-GLP
- Reliability 2 (according to the registration dossier, however only summary available)
- *Positive and negative control groups and treatment:*
    - *Negative control :* yes
    - *Solvent control :* no
    - *Positive control :* no positive control data provided in publication
- *Criteria for evaluating results:* not specified

Test substance

- 1-nitropropane
- *Degree of purity:* 97.4 %
- *Impurities:* 2-nitropropane (2.3 %)

Administration/exposure

- *Strain or cell type or cell line:* Chinese Hamster Lung cells (V79)
- *Target gene:* HPRT
- *Type and composition of metabolic activation system:* no

- *Test concentrations:* 0, 0.3, 1, 3, 6 and 10 mM (= limit dose). Limited cytotoxicity was observed at the highest concentration tested (+20 %).
- *Treatment time:* 3 h
- *Vehicle:* medium
- *Statistical methods:* not specified

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* yes. Marginally cytotoxic (relative percent survival was approximetaly 80 % at 3 and 10 mM and 95 % at 0.3 and 1 mM)
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* positive. The mutation frequency was 11, 28, 31, 53 and 46 x10^6, resp. at 0, 0.3, 1, 3 and 10 mM.
- 1-Nitropropane induced an increase in the number of 6-thioguanine resistant mutants and was thus mutagenic in V79 cells.
- *Remark :* It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations cells have actually been exposed.

3.8.1.3.5 *In vitro* micronucleus test in mammalian cells (Roscher E. *et al.*, 1990)

Study reference:

Roscher E., Ziegler-Skylakakis K. and Andrae U., 1990. Involvement of Different Pathways in the Genotoxicity of Nitropropanes in Cultured Mammalian Cells, Mutagenesis, 5, 375-380.

Detailed study summary and results:

Test type

- Similar to OECD TG 487 (according to registration dossier, however only summary available, not enough information to confirm the validy of the study)
- Non-GLP
- Reliability 2 (according to the registration dossier, however only summary available)
- *Positive and negative control groups and treatment:*
    - *Negative control :* yes
    - *Solvent control :* no
    - *Positive control :* no positive control data provided in publication
- Scoring of 3 x 500 cells per slide
- *Criteria micronucleus:*
    - fluorescence similar to that of the nucleus
    - size up to 25 % of the nuclear area and
    - distinct separation from the nucleus, were regarded as micronuclei.

- Cells containing up to four micronuclei were considered as micronucleated. Cells with (i) more than four micronuclei, (ii) several nuclei of similar size or (iii) heavily fragmented nuclei were regarded as multinucleated.

Test substance

- 1-nitropropane
- *Degree of purity:* 97.4 %
- *Impurities:* 2-nitropropane (2.3 %)

Administration/exposure

- *Strain or cell type or cell line:* Chinese Hamster Lung cells (V79)
- *Type and composition of metabolic activation system:* no
- *Test concentrations:* 0, 0.3, 1, 3, 6 and 10 mM (= limit dose). Limited cytotoxicity was observed at the highest concentration tested (+20 %).
- *Treatment time:* 3 h
- *Vehicle:* medium
- *Statistical methods*

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* yes, marginally cytotoxic (relative percent survavival was approximately 80 % at 3 and 10 mM and 95 % at 0.3 and 1 %)
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* positive. The number of micronuclei cells was of 8, 6, 14 and 43 $x10^3$, respectively at 0, 1, 3 and 10 mM
- 1-Nitropropane induced a clear increase in the formation of micronuclei in V79 cells. An increased number of multinucleated cells was also observed.
- *Remark:* It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations cells have actually been exposed.

3.8.1.3.6 *In vitro* DNA damage and/or repair study (Andrae U. *et al.*, 1988)

Study reference:

Andrae U., Homfeldt H., L. Vogl, J. Lichtmannegger and K.H.Summer, 1988. 2-Nitropropane induces DNA repair synthesis in rat hepatocytes in vitro and in vivo, Carcinogenesis, 9(5), 811-815.

Detailed study summary and results:

Test type

- No guideline followed
- Non-GLP
- Reliability 2 (according to the registration dossier, however only summary available)

- It was not specified if the protocol for the *in vitro* unscheduled DNA synthesis assay in mammalian cells was adapted for volatile compounds. However, as the study was part of the study 3.8.1.3, flasks were probably also closed air tight.
- *Positive and negative control groups and treatment:*
  - *Negative control :* yes but no solvent control
  - *Positive control :* yes, methylmethanesulfonate
- *Criteria for evaluating results:* no additional information available

Test substance

- 1-nitropropane
- *Degree of purity:* 97.4 %
- *Impurities:* 2-nitropropane (2.3 %)

Administration/exposure

- *Strain or cell type or cell line:* cell lines of extrahepatic origin derived from
  rat (208F (embryonic fibroblasts) and LLC-WRC 256 (carcinoma Walker rat)),
  mouse (3T3-NIH (embryonic fibroblasts)),
  hamster (V79 (fibroblasts, lung) and CHO (fibroblasts, ovary)) and
  man ((WI38 embronic fibroblasts, lung), NCI-H322 (adenocarcinoma, lung), A549 (adenocarcinoma, lung) and HEp2 (epiderm. carcinoma, larynx)).
- *Type and composition of metabolic activation system:* no
- *Test concentrations:* 0.1-10 mM
- *Statistical methods:* not specified

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* unspecified
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* negative
- 1-Nitropropane did not induce DNA repair in non-hepatic cell lines from rat, mouse, hamster and human.
- *Remark :* No access to manuscript or raw data. Results of *in vitro* UDS tests should be interpreted with caution as this test method is considered obsolete and has been deleted from the OECD TG program.

3.8.1.3.7 *In vitro* gene mutation test in bacteria (Anonymous 33, 1994)

Study reference:

Anonymous 33, 1994

Detailed study summary and results:

Test type

- OECD TG 471
- GLP
- Relibiality 1 (according to the registration dossier)
- *Number of replicates:* Each condition was tested in triplicate.
- *Positive and negative control groups and treatment:*
    - *Negative control :* yes (vehicle control)
    - *Positive control :* with met. act. : 2-aminoanthracene for *S. typh* TA1535 and *E. Coli* WP2uvrA- and benzo(a)pyrene for *S. typh* TA1537, TA98 and TA100

      Without met. act. : N-ethyl-N'-nitro-N-nitroguanidine for *S. typh* TA1535, TA100 and *E. Coli* WP2uvrA-, 9-aminoacridine for *S. typh* TA1537 and 4-nitroquinoline-1-oxide for *S. typh* TA98
- *Criteria for evaluating results:*
    - *Positive :* the compound induced at least a 2-fold, dose-dependent increase in mutation rate (with respect to the spontaneous rate).

Test substance

- 1-nitropropane
- *Degree of purity:* ~99 %
- *Impurities:* other nitroparaffins

Administration/exposure

- *Strain or cell type or cell line:* 4 *S. typh.* strains (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA-
- *Type and composition of metabolic activation system:*
    - *species and cell type:* S9 fraction prepared from liver Sprague-Dawley rats induced with Aroclor1254 (500 mg/kg) (purchased from BIBRA)
    - *quantity:* 0.5 mL 10 % S9 mix/plate
- *Test concentrations:* A range-finding study was performed. As no cytotoxicity was observed for any of the strains with or without metabolic activation, five concentrations up to the max recommended concentration of 5000 µg/plate were tested. Two independent experiments were performed:
    - *First experiment :* 8, 40, 200, 1000 and 5000 µg/plate
    - *Second experiment :* 312.5, 625, 1250, 2500 and 5000 µg/plate
- *Vehicle:* DMSO
- *Statistical methods:* not required

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* no
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* negative

No significant increase in the frequency of revertant colonies was observed for any of the bacterial strains with any concentration in two independent experiments either in presence or in absence of S9 metabolic fraction. Under the test conditions, the compound is therefore considered as non-mutagenic.

Table 25: Number of revertants (number of colonies/plate) (Exp 1)

	Without met. act.					With met. act.				
Conc. (in µg/plate)	TA100	TA1535	TA98	TA1537	WP2uvrA-	TA100	TA1535	TA98	TA1537	WP2uvrA-
0	134.7	12.0	18.3	14.7	24.3	130.7	17.0	28.7	12.7	38.0
8.0	123.3	13.0	16.0	10.3	27.7	125.3	14.3	22.7	12.0	41.7
40	125.3	10.7	12.3	13.7	29.0	132.3	15.7	26.3	13.7	38.0
200	106.3	12.3	14.3	11.0	32.3	134.7	14.3	27.3	12.3	30.0
1000	134.0	12.7	17.3	12.3	26.0	113.7	13.7	15.7	11.3	39.3
5000	121.7	14.3	12.7	10.0	34.7	131.0	15.3	23.7	12.3	32.3
PC	408.3	113.3	116.7	501.0	449.3	514.7	125.3	177.7	145.7	160.0

Table 26: Number of revertants (number of colonies/plate) (Exp 2)

	Without met. act.					With met. act.				
Conc. (in µg/plate)	TA100	TA1535	TA98	TA1537	WP2uvrA-	TA100	TA1535	TA98	TA1537	WP2uvrA-
0	159.3	24.0	26.3	15.7	34.3	149.7	26.0	28.7	12.0	37.0
312.5	139.7	22.7	20.7	10.3	27.7	147.7	18.0	27.3	13.0	33.7
625	141.7	27.3	16.7	13.7	30.3	160.7	18.3	36.3	11.3	33.3
1250	148.7	24.0	20.3	11.7	35.3	143.3	21.7	24.3	12.7	27.0
2500	149.7	31.3	19.0	14.3	37.3	155.7	22.7	31.0	11.7	26.3
5000	157.0	23.0	20.0	12.7	37.3	153.7	30.0	30.7	13.3	37.7
PC	518.3	168.3	149.7	489.3	589.0	479.0	144.7	180.3	99.7	165.0

- *Remark :* It should be noted that the protocol was not adapted for volatile compounds and consequently, it is not clear to which concentrations bacteria have actually been exposed.

3.8.1.3.8 *In vitro* gene mutation test in bacteria (Haworth S. *et al., 1983)*

Study reference:

Haworth S., Lawlor T., Mortelmans K., Speck W. and Zeiger E., 1983. Salmonella mutagenicity tests. II. Results for 250 chemicals, Environ Mutagen (suppl. 1), 3-142.

Detailed study summary and results:

Test type

- Not enough information available to confirme the similarity to the OECD TG 471
- Non-GLP
- Reliability 1 (according to the registration dossier, however not enough information to confirm the validity of the study, and 4 strains instead of 5)
- *Number of replicates:* All tests were run in triplicate.
- *Positive and negative control groups and treatment:*
    - *Negative control :* no
    - *Solvent control :* yes
    - *Positive control :* with met. act. : 2-aminoanthracene (2-AA)

      Without met. act. : 4-nitro-o-phenylenediamine (NOPD) for TA98, sodium azide (SA) for TA100 and TA1535 and 9-aminoacridine (9-AAD) for TA1537
- *Criteria for evaluating results:*
    - *Positive :* dose-related increase in the number of revertants with respect to the negative control (even if the increase was less than two fold);
    - *Negative :* no increase in the number of mutants;
    - *Questionable :* no clear dose response relationship, no reproducible dose-related relationship, or if the response was of insufficient magnitude to support a positive response.

Test substance

- 1-nitropropane
- *Degree of purity:* 97 %
- Coded before testing

Administration/exposure

- *Strain or cell type or cell line:* 4 *S. typh.* strains (TA98, TA100, TA1535 and TA1537)
- *Type and composition of metabolic activation system:*
    - *species and cell type:* S9 fraction prepared from liver of Sprague-Dawley rats or Syrian hamsters induced with Aroclor 1254
    - *quantity:* 10 %
- *Test concentrations:* 100, 333, 1000, 3333 and 10000 µg/plate. No cytotoxicity was observed at the highest concentration tested. No precipitation was present in any of the test conditions.
- *Vehicle:* DMSO
- *Statistical methods:* not applicable

Results and discussion

- *Cytotoxic concentrations with and without metabolic activation:* no
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* negative

No significant increase in the frequency of revertant colonies was observed for any of the bacterial strains with any concentration either in presence or in absence of S9 metabolic fraction. Under the test conditions, the compound is therefore considered as non-mutagenic.

Table 27: 1-nitropropane (CWR)

	TA100			TA1535			TA1537			TA98		
Doses	NA	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI
0	101	140	140	7	10	9	3	10	10	20	28	24
100	110	162	202	7	7	7	4	10	10	12	22	21
333	127	157	148	7	13	8	9	7	8	14	19	17
1000	101	188	168	5	8	9	8	9	11	16	22	21
3333	100	145	138	8	8	11	7	6	11	12	19	24
9770	96	120	103	4	9	16	4	11	6	14	31	23
PC	546	2385	2642	259	78	107	307	24	98	250	2369	2294

CWR : Case Western reserve University; NA : no activation; RLI : rat liver S-9; HLI : hamster liver S9; PC : positive control

Table 28: 1-nitropropane (CWR)

	TA100			TA1535			TA1537			TA98		
Doses	NA	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI
0	147	275	280	5	7	8	2	6	7	15	24	23
100	152	252	252	4	5	5	2	3	6	11	20	19
333	197	244	276	6	6	4	3	5	4	12	18	22
1000	144	268	257	4	8	7	3	7	5	16	20	22
3333	127	226	228	6	5	9	3	5	5	14	20	21
9770	135	142	157	4	7	9	2	6	4	15	27	22
PC	715	2796	2383	351	122	86	503	119	116	243	1163	1528

CWR : Case Western reserve University; NA : no activation; RLI : rat liver S-9; HLI : hamster liver S9; PC : positive control

Table 29: 1-nitropropane (SRI)

	TA100			TA1535			TA1537			TA98		
Doses	NA	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI
0	85	110	120	5	9	5	3	4	5	25	25	28
100	93	115	117	6	3	4	4	5	4	29	25	19
333	90	118	140	7	6	4	4	5	5	19	22	28
1000	83	104	116	6	8	4	5	4	5	25	27	22
3333	100	116	117	8	4	5	5	4	4	21	24	20
10000	5	82	52	2	0	0	3	3	2	4	10	19

PC	233	1938	2236	154	542	530	216	231	89	619	1465	2152

SRI : SRI international; NA : no activation; RLI : rat liver S-9; HLI : hamster liver S9; PC : positive control

Table 30: 1-nitropropane (SRI)

	TA100			TA1535			TA1537			TA98		
Doses	NA	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI	NA	RLI	HLI
0	121	117	145	7	7	5	5	5	6	13	16	19
100	124	119	185	15	6	7	6	5	4	13	15	19
333	124	123	181	13	5	7	5	4	6	13	16	23
1000	140	123	150	10	7	6	3	6	5	12	14	16
3333	140	136	150	14	5	11	3	4	5	11	17	21
10000	t	t	0	t	t	11	t	t	2	t	t	6
PC	275	1916	1888	231	224	614	261	77	204	718	634	875

SRI : SRI international; NA : no activation; RLI : rat liver S-9; HLI : hamster liver S9; PC : positive control; t : complete clearing of background lawn

- *Remark :* It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations bacteria have actually been exposed.

3.8.2 Animal data

3.8.2.1 *In vivo* data on NITROMETHANE

3.8.2.1.1 *In vivo* micronucleus test (NTP, 1997)

Study reference:

National Toxicology Program. 1997. Toxicology and carcinogenesis studies of nitromethane (CAS No. 75-52-5) in F344/N rats and B6C3F1 mice. National Toxicology Program Technical Report Series No. 461. U.S. Department of Health and Human Services (USDHHS), Public Health Service, National Institute of Health (NIH), NIH Publication No. 97, 3377.

Detailed study summary and results: An *in vivo* micronucleus test in normochromatic erythrocytes (NCEs) of B6C3F1 mice was performed. After 13-week exposure to nitromethane vapour, peripheral blood samples were obtained and prepared as described in MacGregor *et al.*, 1983. Frequency of micronuclei was determined in 2000 NCEs from each animal in each dose group. After 13-week of exposure, nitromethane did not induce an increase in frequency of micronucleated erythrocytes from peripheral blood.

Test type

- Equivalent to OECD TG 474
- Non-GLP
- Reliability 2 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* >98 %

Test animals

- *Species/strain/sex:* Mice / B6C3F1 / both sexes
- *Nb. of animals per sex per dose:* 10/sex/dose

Administration/exposure

- *Doses/concentration levels:* 0, 94, 188, 375, 750 and 1500 ppm (=limit dose)
- *Vehicle:* not specified
- *Details on test system and conditions, and details on route of administration, exposure:*
    - inhalation (vapour)
- *Duration of study, frequency of treatment, sampling times and number of samples:*
    - 6 h/d
    - 5 d/week for 13 weeks
- *Positive and negative (vehicle/solvent) control data:*
    - Concurrent vehicle control
    - *Positive control:* URNE (according to NTP, not identified)
- *Criteria for scoring and number of cells analysed per animal:*
    - frequency of micronuclei in 2000 normochromatic erythrocytes (NCEs) in each animal in each dose group
    - Criteria of Schmid (1976) + micronuclei exhibit the characteristic fluorescent emissions of DNA + the minimum size limit was approximately one-twentieth the diameter of the NCE cell.
- *Statistical methods:* increasing trend over exposure groups was evaluated with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure group and the control group (Margolin *et al.*, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation.
- *Evaluation criteria:*
    - *Positive:* the trend test P value is less than or equal to 0.025 or the P value for any exposure group is less than or equal to 0.025 divided by the number of exposed groups.

Results and discussion

- *Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal):* No increase in the frequencies of micronucleated erythrocytes was observed in the peripheral blood of male or female mice that had been administered nitromethane by inhalation for 13 weeks at concentrations up to 1500 ppm.

Table 31: Frequency of micronucleated erythrocytes from mice peripheral blood after a 13-week inhalation treatment of nitromethane

Dose level (ppm)	Sex	0	94	188	375	750	1500	Trend test*
% of micronucleated NCEs (mean ± St.Dev)	Males	0.052 ± 0.0076	0.080 ± 0.0078	0.061 ± 0.0064	0.067 ± 0.0111	0.064 ± 0.0076	0.070 ± 0.0066	P = 0.273
	Females	0.055 ± 0.0071	0.037 ± 0.0062	0.040 ± 0.0068	0.0.39 ± 0.0031	0.055 ± 0.0056	0.049 ± 0.0064	P = 0.186

*statistical significance tested by one-tailed trend test, significant if P < 0.025

- *Remark:* Based on the information provided, it is not clear whether nitromethane reached the bone marrow. However, the compound was tested up to the limit dose and no effects was observed in the *in vitro* chromosome aberration and micronucleus test.

3.8.2.1.2 Other studies

Gocke *et al.* study (*in vivo* micronucleus test and *in vivo* gene mutation tests) was mentioned by the registrant in the registration dossier and the full study report was made available to the DS. However, due to very poor quality of the copy, the study will not be presented in the CLH report and will not be assessed.

3.8.2.2 *In vivo* data on NITROETHANE

3.8.2.2.1 *In vivo* micronucleus test (Hite and Skeggs, 1979)

Study reference:

Hite M and Skeggs H., 1979. Mutagenic evaluation of nitroparaffins in the *Salmonella typhimurium*/ mammalian-microsome test and the micronucleus test. Environ Mutagen, 1, 383-389.

Detailed study summary and results:

Test type

- *In vivo* micronucleus test in polychromatic erythrocytes of CD-1 mice
- Prior to OECD TG 474
- Prior to GLP
- Reliability 2 (according to the registration dossier, however full study report not available, the deviations to the OECD TG 474 could not be checked)

Test substance

- Nitroethane
- *Degree of purity:* not specified

Test animals

- *Species/strain/sex:* Mice / Charles River (CD-1) / both sexes
- *Nb. of animals per sex per dose:*
  - *Controls:* 14 males + 14 females

- *Dose groups:* 8 males + 8 females

Administration/exposure

- *Doses/concentration levels:* 0.25, 0.5 and 1.00 mL/kg bw/day. The highest dose corresponded to half of the oral LD50 value.
- *Vehicle:* not specified
- *Details on test system and conditions, and details on route of administration, exposure*
  - Oral (gavage)
- *Duration of study, frequency of treatment, sampling times and number of samples*
  - twice a day
  - Animals were sacrificed 6h after the last dose
- *Positive and negative (vehicle/solvent) control data*
  - Concurrent control (tap water - 1.0 mL/kg/day)
  - Positive control: methylmethanesulfonate (90 mg/kg bw/day i.p.)
- *Criteria for scoring and number of cells analysed per animal:* Frequency of micronuclei in 3000 eryhrocytes in each animal per dose group
- *Statistical methods:* analysis of variance procedure using a rankit transformation of the data and Fisher's Least Significance Difference procedure for joint comparison between the negative control and a treated group.
- *Evaluation criteria:* No additional information

Results and discussion

- *Effect on mitotic index or PCE/NCE (polychromatic erythrocyte/normochromatic erythrocyte) ratio by dose level by sex (if applicable):*

Table 32: Induction of polychromatic erythrocytes (PCEs) with micronuclei by nitroethane

Test condition	Sex (number)	% PCEs with micronuclei
0 (tap water)	Female (14)	0.64
	Male (14)	0.53
	Combined (28)	0.58
0.25 mL/kg/day, p.o.	Female (8)	0.44
	Male (8)	0.51
	Combined (16)	0.48
0.50 mL/kg/day, p.o.	Female (8)	0.47
	Male (8)	0.67
	Combined (16)	0.57
1.0 mL/kg/day, p.o.	Female (8)	0.57
	Male (8)	0.60
	Combined (16)	0.59

Positive control – methyl methanesulfonate (90 mg/kg/day, i.p.)	Female (5)	6.09*
	Male (5)	5.76*
	Combined (10)	5.92*

- *Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal):* In contrast to the positive control compound, nitroethane did not induce a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes of male or female mice at doses up to 1.00 mL/kg bw/day.
- Based on the available information, it is not clear whether nitroethane reached the bone marrow. Consequently, the negative result of this *in vivo* micronucleus test should be interpreted with caution, especially as no *in vitro* data of chromosome aberration or micronucleus tests were provided by the applicant.

3.8.2.3 *In vivo* data on 1-NITROPROPANE

3.8.2.3.1 *In vivo* micronucleus test (George E. *et al.*, 1989)

Study reference:

George E, Burlinson B and Gatehouse D., 1989. Genotoxicity of 1- and 2-nitropropane in the rat, Carcinogenesis, vol.10, 2329-2334.

Detailed study summary and results:

Test type

- No guideline followed
- Non-GLP
- Reliability 2 (according to the registration dossier, however no access to raw data)

Test substance

- 1-nitropropane
- *Degree of purity:* no details on the identity of the test substance were provided.

Test animals

- *Species/strain/sex:* rat / SD / male
- *Nb. of animals per sex per dose:* 4-8 rats/group
- *Age and weight at the study initiation:* 8-12 w

Administration/exposure

- *Doses/concentration levels:*
  - *Bone marrow :*
    - 24 h : 100, 200, 300 and 400 mg/kg
    - 48 h : 100, 200 and 300 mg/kg
  - *Liver :*

- 72 h : 300 mg/kg
- Comment : lethality observed at 500 mg/kg

- *Vehicle:* water
- *Details on test system and conditions, and details on route of administration, exposure*
  - Oral (gavage)
- *Duration of study, frequency of treatment, sampling times and number of samples:*
  - Single dose
  - Animals were sacrificed 24 h or 48 h (bone marrow) or 72 h (liver) after dosing
- *Positive and negative (vehicle/solvent) control data*
  - *Negative control :* Concurrent vehicle
  - *Positive control:*
    - Bone marrow: cyclophosphamide
    - Liver: 4-acetylaminofluorene
- *Criteria for scoring and number of cells analysed per animal:*
  - Coded slides
  - Bone marrow: Frequency of micronuclei in 2000 polychromatic erythrocytes per slide
  - Liver: Frequency of micronuclei in 2000 hepatocytes per slide
- *Statistical methods:*
  - Bone marrow: Data were analyzed according to methods published by Amphlett and Delow (Mut. Res. 128:161-164).
  - Liver: Student's t-test or analysis of variance.
- *Evaluation criteria :* no information

Results and discussion

- *Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal):* negative in the bone marrow, however positive in liver
- *Toxicity :* yes, lethality at 500 mg/kg
- *Describe additional information:*
  - *Bone marrow:*
    - Slight reduction in the percentage of PCE for both sampling time
    - Small dose-related increase in the frequency of micronucleated cells compared to controls :

Table 33: Incidence of micronuclei and PCE

Experiment	A									B			
Sampling time	24 h					48 h				24 h			
Dose (in mg/kg)	0	100	200	300	PC	0	100	200	300	0	300	400	PC
Nb. animals tested	6	6	6	6	4	6	6	6	6	3	5	5	3

MN PCE/1000 PCE	0.83	1.00	1.42	1.58*	8.40*	0.92	1.17	1.08	1.83	1.33	1.70	1.50	8.33*
% PCE	34.0	30.6	31.4	28.1	24.7	39.9	33.4	34.4	28.0	39.1	44.1	43.4	35.8

2000 PCE analysed for micronucleus frequency; 500 erythrocytes for %

At 24h : 1.58 MN PCE/1000 PCE at 300 mg/kg compared to 0.83 MN PCE/1000 PCE in control group (positive control : 8.4 MN PCE/1000 PCE)

At 48h : 1.83 MN PCE/1000 PCE at 300 mg/kg compared to 0.92 MN PCE/1000 PCE in control group.

- *Liver:*
  - Significant increase in the frequency of micronuclei in hepatocytes :

    17.05 micronucleated cells/1000 hepatocytes in treated animals compared to 7.34 micronucleated cells/1000 hepatocytes in control group. Which was accompanied by an increased mitotic index (28.85 mitoses/1000 hepatocytes vs 14.92 mitoses/1000 hepatocytes).

    14.20 micronucleated cells/1000 hepatocytes in treated animals compared to 5.03 micronucleated cells/1000 hepatocytes.

- *Discuss if it can be verified that the test substance reached the general circulation or target tissue, if applicable:* It is not clear whether 1-nitropropane reached the bone marrow.
- Nitropropane was negative in the *in vivo* micronucleus test in bone marrow but induced an increase in the micronuclei frequency in hepatocytes which was assigned to increased cell proliferation.
- *Remarks:* No access to manuscript or raw data.

3.8.2.3.2 *In vivo* mammalian cell study : DNA damage and/or repair (Andrae U. *et al.*, 1988)

Study reference:

Andrae U., Homfeldt H., Vogl L., Lichtmannegger J. and Summer K.H., 1988. 2-Nitropropane induces DNA repair synthesis in rat hepatocytes *in vitro* and *in vivo*, Carcinogenesis, vol 9, no.5, 811-815.

Detailed study summary and results:

Test type

- No guideline followed
- Not-GLP
- Reliability 2 (according to the registration dossier, however no access to raw data)

Test substance

- 1-nitropropane
- *Degree of purity:* 97.4 %
- *Impurities:* 2-nitropropane (2.3 %)

Test animals

- *Species/strain/sex:* rat / Wistar / both sexes
- *Nb. of animals per sex per dose:* 9 controls and 2 rats/sex after 1 and 17 h
- *Age and weight at the study initiation:* 150-200 g

Administration/exposure

- *Doses/concentration levels:* 0, 20, 40, 60 and 80 mg/kg bw
- *Vehicle:* olive oil
- *Details on test system and conditions, and details on route of administration, exposure:* IP
- *Duration of study, frequency of treatment, sampling times and number of samples*
    - Single exposure
    - 1.5 h
- *Positive and negative (vehicle/solvent) control data*
    - *positive control :* dimethylnitrosamine and methylmethanesulfonate

Results and discussion

- *Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal):* negative, "the test substance did not cause increase repair synthesis in males treated with 20 – 80 mg/kg for 4 h but did slightly reduce the repair background. Likewise, no repair induction was observed when male rats were injected with 60 mg/kg and killed 1 h or 17 h later. 1-nitropropane was also ineffective in inducing repair in HPC from female rats treated *in vivo*"

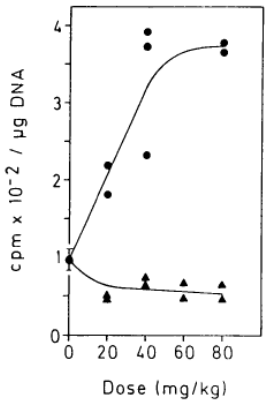

Fig. 3. Induction of DNA repair synthesis in HPC from male rats treated with 2-NP or 1-NP *in vivo*. Animals were injected i.p. with 2-NP or 1-NP and killed 4 h later. Hepatocytes were isolated and allowed to attach in the presence of FdUrd and BrdUrd for 1.5 h. Subsequently HPC were exposed to [^{3}H]dCyd (10 µCi/ml), FdUrd and BrdUrd for 20 h. Repair synthesis was determined as described in Materials and methods. Each data point represents the results from one animal. ●, 2-NP; ▲, 1-NP.

3.8.2.3.3 *In vivo* mammalian somatic cell study: cytogenicity/erythrocyte micronucleus (Kliesch U. and Adler I.D., 1987)

Study reference:

Kliesch U. and Adler I.D., 1987. Micronucleus test in bone marrow of mice treated with 1-nitropropane, 2-nitropropane and cisplatin, Mutation Research, 192, 181-184.

Detailed study summary and results:

Test type

- No guideline followed
- GLP: unspecified
- Reliability 2 (according to the registration dossier. However, article mostly not readable due to the bad quality of the PDF file)

Test substance

- 1-nitropropane
- *Degree of purity:* not specified

Test animals

- *Species/strain/sex:* mouse / strain not specified / both sexes
- *Nb. of animals per sex per dose:* 5/sex/group
- *Age and weight at the study initiation:* 12-14 w of age

Administration/exposure

- *Doses/concentration levels:* no information available
- *Vehicle:* physiological saline
- *Details on test system and conditions, and details on route of administration, exposure:* IP
- *Duration of study, frequency of treatment, sampling times and number of samples:*
    - Single injection
- *Positive and negative (vehicle/solvent) control data:* no information available
- *Post-exposure peiord:* 24 and 72 h

Results and discussion

- *Effect on mitotic index or PCE/NCE (polychromatic erythrocyte/normochromatic erythrocyte) ratio by dose level by sex (if applicable):* "the highest frequency of micronucleated polychromatic erythrocytes was 0.26 %, not statistically elevated from the control group. Experiments with 2-Nitropropane or 1-Nitropropane did not reveal any clastogenic activity of the compounds. No dose- or time-dependent increase in the number of PCE were found. There was no increase in micronucleus rates at 300 mg/kg bw at either 24 or 72 hours. A single positive finding at 200 mg/kg bw was not reproducible or dose-dependent."
- *Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal):* negative
- *Concurrent positive control data:* no information available
- *Statistical results:* no information available

3.8.3 Human data

No human data available regarding nitromethane, nitroethane and 1-nitropropane

3.8.4 Other data

No other data available regarding nitromethane, nitroethane and 1-nitropropane

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 Animal data on NITROMETHANE

3.9.1.1.1 2-year repeated dose toxicity study in rats, by inhalation (NTP, 1997)

Study reference:

National Toxicology Program. 1997. Toxicology and carcinogenesis studies of nitromethane (CAS No. 75-52-5) in F344/N rats and B6C3F1 mice. National Toxicology Program Technical Report Series No. 461. U.S. Department of Health and Human Services (USDHHS), Public Health Service, National Institute of Health (NIH), NIH Publication No. 97-3377.

Detailed study summary and results:

Test type

- Guideline 451 FDA
- GLP-compliant
- Reliability 1 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* 99.3 % (lot 1-H-0501, batch 2, used at the beginning of the 2-year study), 99 % (lot 1-H-1004, used for the remainder of the 2-year study)
- *Impurities:* lot 1-H-0501, batch 2 : nitroethane 0.27 %; lot 1-H-1004 : nitroethane (0.25 %) and 2-nitropropane (0.03 %). The presence of 2-nitropropane does not affect the classification.

Test animals

- *Species/strain/sex:* Rat / Fischer F344/N / males and females
- *Nb. of animals per sex per dose:* 50
- *Age and weight at the study initiation:* 7 weeks – males: ~ 145 g, females: ~ 116 g

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *duration of test/exposure period:* 2 y
- *doses/concentration levels:* 0 ppm , 94 ppm, 188 ppm, 375 ppm : based on a 13-week range-finding study where a severe degeneration of the sciatic nerve and spinal cord was observed in rats exposed to 750 or 1500 ppm. These changes were considered as minimal in the 375 ppm groups.
- *frequency of treatment:* 6 hours 12min/day, 5 days/week
- *control group and treatment:* control + 3 doses

- *historical control data:* yes
- *post exposure observation period:* no
- *type of inhalation exposure and test conditions (e.g.: exposure apparatus):* nitromethane was held in a stainless-steel reservoir under a nitrogen blanket; a Master-Flex variable-speed peristaltic pump head was used to pump nitromethane through a liquid distribution manifold of stainless steel tubing to heated-wick vaporizers. One set of dual vaporizers supplied nitromethane vapour to all chambers.
- *method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* "whole body", exposure data : buildup and decay rates for chamber concentrations were determined with and without animals present in the chambers.
- *analytical verification of test atmosphere concentrations:* "whole body", exposure data : buildup and decay rates for chamber concentrations were determined with and without animals present in the chambers.

Results and discussion

- *Mortality and time to death (indicate number died per sex per dose and time to death):* mortality was relatively high in all groups but was not dose-related

Table 34: Mortality rate in male and female rats

Dose level (ppm)	0	94	188	375
Males (%)	37/50 (74)	34/50 (68)	36/50 (72)	42/50 (84)
Females (%)	22/50 (44)	31/50 (62)	20/50 (40)	27/50 (54)

- *Clinical signs:* masses on shoulder and torso consistent with mammary gland neoplasms were observed in females in the 188 and 375 ppm groups but no other treatment-related clinical findings were observed
- *Body weight gain:* no effects in male, slightly greater than controls in females exposed to 375 ppm

Table 35: Mean BW (g) in rats and relative BW compared to controls (%)

Dose level (ppm)		0	94	188	375
In males					
Weeks	1-13	270	271 (100)	269 (100)	266 (99)
	14-52	455	456 (100)	454 (100)	458 (101)
	52-103	514	514 (100)	496 (96)	518 (101)
In females					
Weeks	1-13	163	165 (101)	165 (101)	163 (100)
	14-52	247	251 ((102)	255 (103)	261 (106)
	52-103	341	345 (101)	354 (104)	360 (106)

- *Food/water consumption:* no data
- *Ophthalmoscopic examination:* no data
- *Clinical chemistry:* no data for the long-term study. Clinical chemistry was analysed in the 13 week-range finding study where a "transient change in thyroid hormone concentrations was observed (hypothyroid state, evidenced by decresed serum triiodotnyronine, thyroxine, and free thyroxine on day 23 in males exposed to 375 ppm or greater and females exposed to 750 or 1500 ppm) but at 13 weeks, hormone concentrations of exposed rats were similar to those of the controls."
- *Haematology:* no data for the long-term study. Hematology was analysed in the 13 week-range finding study where a "concentration-dependent, microcytic, anemia" was observed.
- *Urinalysis:* no data
- *Organ weights:* no data
- *Necropsy findings:* tumours of mammary glands were observed in females exposed.
- *Histopathological findings:*
    - *mammary gland:* in females, the incidences of fibroadenoma, fibroadenoma or adenoma (combined) and fibroadenoma, adenoma or carcinoma (combined) increased in a dose-dependent manner
    - *kidney:* in males, hyperplasia was observed in renal tubule in 6, 8, 6 and 12 out of 50 males at necropsy, at 0, 94, 188 and 375 ppm, respectively. Severity was mild to moderate in control and low dose groups and mild in middle and high dose groups. Adenomas were also reported in renal tubule of 2, 5, 2 and 5 out of 50 males, at 0, 94, 188 and 375 ppm, respectively. Those effects were not dose-related or statistically significant.
- *Tumour incidence data by sex, dose and tumour type:*
    - *males :* no tumours observed
    - *females :* mammary glands: data for 0 ppm, 94 ppm, 188 ppm, 375 ppm
        - adenoma: 2/50 (4 %), 0/50 (0 %), 0/50 (0 %), 2/50 (4 %)
        - fibroadenoma: 19/50 (38 %), 21/50 (42 %), 33/50 (66 %), 36/50 (72%)
        - carcinoma: 2/50 (4 %), 7/50 (14 %), 1/50 (2 %), 11/50 (22 %)
        - adenoma, fibroadenoma or carcinoma :

          21/50 (42 %), 25/50 (50 %), 35/50 (70 %), 41/50 (82 %)

      Historical controls (same laboratory):

      Carcinoma : F: 0-8 %

      Adenoma, fibroadenoma or carcinoma : F: 22-46 %

Table 36: Incidence of tumours in males and females rats

Dose exposure level (ppm)		**0**	**94**	**188**	**375**	**HCD**
Males	No tumours reported					
	Adenoma (%)	2/50 (4)	0/50	0/50	2/50 (4)	-

Females	Fibroadenoma (%)	19/50 (38)	21/50 (42)	33/50* (66)	36/50* (72)	-
	Carcinoma (%)	2/50 (4)	7/50 (14)	1/50 (2)	11/50 (22)	0-8 %
	Adenoma, fibroadenoma and carcinoma (%)	21/50 (42)	25/50 (50)	35/50* (70)	41/50* (82)	22-46 %

In female rats, the incidence of fibroadenoma, fibroadenoma or adenoma, and fibroadenoma, adeoma or carcinoma was dose-dependent and incidences at the middle and high doses were statistically significant.

- *Local or multi-site responses:* local
- *Progression of lesions to malignancy:* yes
- *Gender and/or species-specific responses:* female response only
- *Mode of action (genotoxic, non-genotoxic):* non-genotoxic but the mode of action has not been elucidated
- *Toxic response data by sex and dose:* /
- *Tumour latency:*

Table 37: First incidence (in days) of mammary glands tumours in females:

Dose exposure level (ppm)	0	94	188	375
Fibroadenoma	454	435	468	552
Carcinoma	631	588	440	425
Fibroadenoma, adenoma or carcinoma	454	435	440	425

- *Statistical methods and results:* logistic regression test

Table 38: Logistic regression test results in females

Dose exposure level (ppm)	0	94	188	375
Fibroadenoma	P<0.001	P=0.219	P=0.003	P<0.001
Carcinoma	P=0.009	P=0.052	P=0.447 N	P=0.011
Fibroadenoma, Adenoma or Carcinoma	P<0.001	P=0.112	P=0.006	P<0.001

Interpretation: in the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by N.

3.9.1.1.2 2-year repeated dose toxicity study in mice (NTP, 1997)

Study reference:

National Toxicology Program. 1997. Toxicology and carcinogenesis studies of nitromethane (CAS No. 75-52-5) in F344/N rats and B6C3F1 mice. National Toxicology Program Technical Report Series No. 461. U.S. Department of Health and Human Services (USDHHS), Public Health Service, National Institute of Health (NIH), NIH Publication No. 97-3377.

Detailed study summary and results:

Test type

- Guideline 451 FDA
- GLP-compliant
- Reliability 1 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* 99.3 % (lot 1-H-0501, batch 2), 99 % (lot 1-H-1004)
- *Impurities:* lot 1-H-0501, batch 2 : nitroethane 0.27 %; lot 1-H-01004 : nitroethane (0.25 %) and 2-nitropropane (0.03 %). The presence of 2-nitropropane does not affect the classification.

Test animals

- *Species/strain/sex:* Mice / B6C3F1 / males and females
- *Nb. of animals per sex per dose:* 50/sex/dose
- *Age and weight at the study initiation:* 7 weeks, males : ~ 25 g, females : ~ 19 g

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration of test/exposure period:* 2 y
- *Doses/concentration levels:* 0 ppm , 188 ppm, 375 ppm, 750 ppm : based on a 13-week range-finding study where extended and severe nasal lesions and splenic hematopoiesis were observed in the 1500 ppm group.
- *Frequency of treatment:* 6 hours12min/day, 5 days/week
- *Control group and treatment:* control + 3 doses
- *Historical control data:* yes
- *Post exposure observation period:* no
- *Type of inhalation exposure and test conditions (e.g.: exposure apparatus):* nitromethane was held in a stainless-steel reservoir under a nitrogen blanket; a Master-Flex variable-speed peristaltic pump head was used to pump nitromethane through a liquid distribution manifold of stainless steel tubing to heated-wick vaporizers. One set of dual vaporizers supplied nitromethane vapour to all chambers.
- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* "whole body"*, exposure data :* buildup and decay rates for chamber concentrations were determined with and without animals present in the chambers.

- *Analytical verification of test atmosphere concentrations:* chamber concentrations were monitored with an on-line gas chromatograph.

Results and discussion

- *Mortality and time to death (indicate number died per sex per dose and time to death):* no treatment-related effect. The survival rate of females in the 750 ppm group was marginally greater than that of the controls.

Table 39: Mortality rate in male and female mice exposed by inhalation to nitromethane

Exposure level (ppm)	0	188	375	750
Male (%)	19/50 (38)	14/50 (28)	20/50 (40)	21/50 (42)
Female (%)	25/50 (50)	22/50 (44)	24/50 (48)	14/50 (28)

- *Clinical signs:* "swelling around the eyes and exophthalmos in exposed males and females. These findings were coincident with harderian gland neoplasms."
- *Body weight gain:* Males : no effects. Females : "The mean body weights of exposed females were generally slightly greater than the mean body weights of the controls during the study but were generally similar to the mean body weight of the control females at the end of the study".

Table 40: Mean body weights (g) in mice

Dose level (ppm)		0	94	188	375
In males					
Weeks	1-13	31.2	30.4	31.4	31.6
	14-52	44.7	43.5	43.8	45.2
	52-103	50.6	49.8	50.5	51.2
In females					
Weeks	1-13	25.1	25.7	26.3	26.3
	14-52	38.2	40.5	40.3	40.8
	52-103	51.3	52.4	51.3	52.4

- *Food/water consumption:* no data
- *Ophthalmoscopic examination:* no data
- *Clinical chemistry, haematology and urinalysis:* no data
- *Organ weights:* no data
- *Necropsy findings:* no findings reported
- *Histopathological findings:* "Nasal lesions were generally greater in exposed male and female mice than those in the controls."

Table 41: Histopathological findings in mice

Dose level exposure (ppm)		0	188	375	750
O.E. degeneration	Males	0/50	10/49**	50/50**	50/50**
	Females	0/50	22/49**	50/50**	50/50**
O.E. metaplasia	Males	0/50	1/49	41/50**	49/50**
	Females	0/50	2/49	46/50**	48/50**
R.E. hyaline degeneration	Males	5/50	5/49	50/50**	50/50**
	Females	16/50	39/49**	50/50**	50/50**

O.E.: olfactory epithelium; R.E.: respiratory epithelium

- *Tumour incidence data by sex, dose and tumour type:* as reported in the study, for harderian glands, adenoma, carcinoma and adenoma or carcinoma rates were similar throughout the study and at termination (overall rate v.s. terminal rate of tumours), in both sexes.

  For the liver tumours, in females, overall and terminal rates were slightly different in adenoma rates (28 – 36, 51 – 61, 35 -38 and 70 – 81 %, for overall – terminal rates, at 0, 188, 375 and 750 ppm, respectively) and carcinoma rates (20 – 12, 29 – 21, 16 – 23 and 24 – 6 %, for overall – terminal rates, at 0, 188, 375 and 750 ppm, respectively).

  For lung tumours, in males, overall and terminal rates were slightly different in adenoma rates at 375 ppm only (18 – 30 % for overall – terminal rates, respectively). The rates were similar at the 0, 188 and 750 ppm for adenomas, and at all doses for carcinomas. For adenoma or carcinoma, overall and terminal rates were slightly different at 375 ppm only (24 – 40 % for overall – terminal rates, respectively). The rates were similar at all the other doses. In females, all rates were similar as we

Table 42: Tumours incidence in the Harderian gland, the liver and the lung of mice exposed for 2 years by inhalation to nitromethane

Dose level exposure (ppm)			0	188	375	750	HCD
Harderian Gland	Adenoma	M (%)	9/50 (18)	10/50 (20)	19/50 (38)	32/50 (65)	-
		F (%)	5/50 (10)	7/50 (14)	16/50 (32)	19/50 (38)	-
	Carcinoma	M (%)	1/50 (2)	1/50 (2)	6/50 (12)	5/50 (10)	M/F:
		F (%)	1/50 (2)	2/49 (4)	4/50 (8)	3/50 (6)	0-4 %
	Adenoma or carcinoma	M (%)	10/50 (20)	11/50 (22)	25/50 (50)	37/50 (74)	2-14 %
		F (%)	6/49 (12)	9/49 (18)	20/50 (40)	21/50 (42)	0-16 %
Liver	Hepatocellular adenoma	M (%)	No effects reported				-
		F (%)	14/50 (28)	25/49 (51)	17/49 (35)	35/50 (70)	-
	Hepatocellular carcinoma	M (%)	No effects reported				-
		F (%)	10/50 (20)	14/49 (29)	8/49 (16)	12/50 (24)	2-30 %
	Hepatocellular adenoma or	M (%)	No effects reported				-
		F (%)	19/50 (38)	34/49 (69)	22/49 (45)	40/50 (80)	6-54 %

	carcinoma						
Lung	Alv / bronch adenoma	M (%)	11/50 (22)	10/50 (20)	9/50 (18)	12/50 (24)	-
		F (%)	3/50 (6)	3/50 (6)	2/49 (4)	9/50 (18)	-
	Alv / bronch carcinoma	M (%)	2/50 (4)	3/50 (6)	3/50 (6)	11/50 (22)	M/F: 0-4 %
		F (%)	0/50 (0)	3/50 (6)	5/49 (10)	3/50 (6)	
	Alv / bronch adenoma or carcinoma	M (%)	13/50 (26)	13/50 (26)	12/50 (24)	20/50 (40)	2-14%
		F (%)	3/50 (6)	6/50 (12)	6/49 (12)	12/50 (24)	0-16 %

Alv/Bronch = alveolar / bronchiolar

- *Local or multi-site responses:* multi-site
- *Progression of lesions to malignancy:* yes
- *Gender and/or species-specific responses:*
  - The liver tumours were only observed in females.
  - Harderian gland : No similar tissue is found in humans.
  - Liver : The spontaneous incidence of liver tumours in this strain of mice is high.
- *Mode of action (genotoxic, non-genotoxic):* non-genotoxic but the mode of action has not been elucidated
- *Toxic response data by sex and dose:* /
- *Tumour latency:* First incidence (days)

Table 43: First incidence (in days) of tumours in male and female mice

Dose level exposure (ppm)			0	188	375	750
Harderian Gland	Adenoma	M	545	448	520	497
		F	609	639	498	503
	Carcinoma	M	653	734 (T)	436	595
		F	663	693	679	734 (T)
	Adenoma or carcinoma	M	545	448	436	497
		F	609	639	498	503
Liver	Hepatocellular adenoma	M	-			
		F	597	534	498	426
	Hepatocellular carcinoma	M	-			
		F	576	534	548	426
	Hepatocellular adenoma or carcinoma	M	-			
		F	576	534	498	426
Lung	Alv / bronch adenoma	M	449	646	734 (T)	497
		F	716	734 (T)	498	426
	Alv / bronch carcinoma	M	734 (T)	734 (T)	734 (T)	586

		F	-	534	602	503
	Alv / bronch adenoma or carcinoma	M	449	646	734 (T)	497
		F	716	534	498	426

(T): terminal sacrifice

- *Statistical methods and results:*

Table 44: Statistical analysis on the Harderian gland tumours

Harderian gland tumours	Dose level (ppm)	0	188	375	750
Fibroadenoma	M	P<0.001	P=0.505	P=0.019	P<0.001
	F	P<0.001	P=0.380	P=0.008	P=0.003
Carcinoma	M	P=0.036	P=0.762 N	P=0.062	P=0.104
	F	P=0.305	P=0.501	P=0.194	P=0.365
Adenoma or carcinoma	M	P<0.001	P=0.506	P=0.001	P<0.001
	F	P<0.001	P=0.175	P=0.002	P=0.002

Interpretation: in the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by N.

Table 45: Statistical analysis on the liver tumours

Liver tumours	Dose level (ppm)	0	188	375	750
Adenoma	M	-			
	F	P<0.001	P=0.013	P=0.364	P<0.001
Carcinoma	M	-			
	F	P=0.329	P=0.195	P=0.383 N	P=0.200
Adenoma or carcinoma	M	-			
	F	P=0.001	P<0.001	P=0.368	P<0.001

Interpretation: in the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by N.

Table 46: Statistical analysis on the lung tumours

Lung tumours	Dose level (ppm)	0	188	375	750
Adenoma	M	P=0.422	P=0.456 N	P=0.412 N	P=0.511
	F	P=0.022	P=0.632 N	P=0.514 N	P=0.083
Carcinoma	M	P=0.001	P=0.569	P=0.485	P=0.009

	F	P=0.149	P=0.119	P=0.033	P=0.110
Adenoma or carcinoma	M	P=0.059	P=0.517 N	P=0.515 N	P=0.105
	F	P=0.007	P=0.243	P=0.238	P=0.015

Interpretation: in the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by N.

3.9.1.1.3 Carcinogenicity study report (Anonymous 34, 1990)

Study reference:

Anonymous 34, 1990

Detailed study summary and results:

Test type

- OECD TG 451
- GLP not specified
- Reliability 1 (according to the registration dossier)
- Major deviations from OECD TG 451 guideline : only 2 doses were tested, 40 animals/group, some tissues were not examined microscopically (parathyroid, epididymis, caecum, rectum, bone marrow,…)
- No data on quality assurance.

Test substance

- Nitromethane
- *Degree of purity:* 96.26 %
- *Impurities*: 2.79 % nitroethane, 0.62 % 2-nitropropane. As 2-nitropropane is classified Carc. Cat. 1B, the presence of this impurity above the generic concentration limit (0.1 %) would lead to a classification as Carc. Cat. 1B.

Test animals

- *Species/strain/sex:* Rat / Long-Evans / both sexes
- *Nb. of animals per sex per dose:* 40
- *Age and weight at the study initiation:* age not provided, weight: males : ~ 166 g, females : ~ 154 g

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration of test/exposure period:* 2 y
- *Doses/concentration levels:* 0, 100 and 200 ppm; rationale for dose level selection : the doses were most probably selected on basis of the occupational exposure limits as it is specified that “the

concentration of 100 ppm is comparable to the Maximum Allowable Exposure published by the U.S. Occupational Safety and Health Administration".

- *Frequency of treatment:* 7 hours/day, 5 days/week
- *Control group and treatment:* 1 control group + 2 doses
- *Historical control data:* no data provided but no tumours observed
- *Post exposure observation period:* no
- *Type of inhalation exposure and test conditions (e.g.: exposure apparatus):* "vapours of nitromethane were generated by bubbling purified nitrogen through liquid nitromethane in an all-glass vessel maintained in a thermostatted water bath at a temperature of 45 °C. Sufficient liquid nitromethane to maintain a constant liquid level in the generator was added automatically."
- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* "whole body", exposure data : the concentration of nitromethane within the chambers was monitored using a MIRAN IA infra-red gas analyser.
- *Analytical verification of test atmosphere concentrations:* Concentrations were measured at least three and usually four times each day.

Results and discussion

- *Mortality and time to death:* no treatment-related effects.

Table 47: Mortality rate

Dose exposure level (ppm)	0	100	200
Males	15/40	17/40	15/40
Females	10/40	11/40	16/40

- *Clinical signs:* no effects
- *Body weight gain:*
  - *males:* similar to controls
  - *females:* significantly lower than controls after 1 year exposure at 100 and 200 ppm
- *Food/water consumption:* not reported
- *Ophthalmoscopic examination:* no data
- *Clinical chemistry:* no clinically significant effects in NA, K, AST, ALT, BUN, PROT and BILI although increases in serum creatinine in both sexes were noted (0.77, 1.01 and 1.26* mg/dL in males and 0.79, 0.75 and 1.17 mg/dL in females, at 0, 100 and 200 ppm, respectively)

Table 48: Serum chemistry findings

Dose level (ppm)	**0**	**100**	**200**
In males			
NA	149.2	149.4	150.0

K	6.33	6.16	6.23
AST	91	47	72
ALT	35	30	38
BUN	17.4	21.1	25.0
CREAT	0.77	1.01	1.26*
PROT	6.18	6.44	6.46
BILI	0.30	0.37	0.37
In females			
NA	148.4	148.3	149.3
K	6.18	5.81	6.05
AST	46	42	51
ALT	29	30	30
BUN	16.1	16.2	22.3
CREAT	0.79	0.75	1.17
PROT	6.88	6.38	6.38
BILI	0.48	0.33	0.32

- *Haematology:* no effects on WBC, RBC, Hb, Htc, MCV, PLT counts after 2 years of exposure, in both sexes

Table 49: Hematological results

Dose level (ppm)	**0**	**100**	**200**
In males			
WBC ($x10^3$)	11.4	9.7	9.0
RBC ($x10^6$)	7.40	7.52	7.19
Hb (g/dL)	13.8	13.9	12.9
Htc (%)	41.6	42.0	39.8
PLT ($x10^3$)	1397	1380	1488
In females			
WBC ($x10^3$)	7.3	9.0	7.3
RBC ($x10^6$)	7.57	7.51	7.42
Hb (g/dL)	14.9	14.6	14.1
Htc (%)	44.6	43.8	42.4
PLT ($x10^3$)	1156	1092	1126

- *Urinalysis:* no data

- *Organ weights:* no effects in either sex (absolute & relative brain, liver, kidneys, lungs and heart weights have been weighed)

Table 50: Organ weights data (absolute and relative: g (%))

Dose level (ppm)	0	100	200
In males			
BW	641	639	631
Brain	2.306 (0.368)	2.392 (0.394)	2.300 (0.370)
Heart	1.949 (0.311)	1.904 (0.303)	2.062 (0.330)
Kidney	4.986 (0.799)	4.948 (0.875)	5.177 (0.830)
Liver	16.044 (2.555)	17.194 (2.776)	16.748 (2.681)
lung	2.532 (0.402)	2.454 (0.395)	2.618 (0.420)
In females			
BW	448	410	417
Brain	2.140 (0.486)	2.086 (0.522)	2.124 (0.527)
Heart	1.513 (0.342)	1.464 (0.366)	1.481 (0.362)
Kidney	3.443 (0.781)	3.304 (0.824)	3.419 (0.854)
Liver	12.828 (2.897)	12.305 (3.038)	11.713 (2.868)
Lung	2.124 (0.482)	2.121 (0.535)	2.251 (0.561)

- *Necropsy findings:* no effects
- *Histopathological findings:* effects were observed in all animals (controls + exposed) but were not treatment-related : bronchitis, glomerulosclerosis, calcification of the kidneys, vacuolation of the adrenal cortex and fibrocystomas in the mammary gland.
- *Tumour incidence data by sex, dose and tumour type:* No treatment-related increase of tumours.
  In all animals, an increase in the incidence of benign tumours (adenoma of the pituitary gland, fibroadenomas and multiple fibroadenomas of the mammary glands) was observed but was similar in control and exposed animals.
  Malign tumours were very rare and no treatment-relationship was observed.

Table 51: Tumours incidence

Dose level (ppm)		0	100	200
In males				
Mammary gland	**Adenocarcinoma**	0	2	0
	Fibroadenoma	0	1	0
	Fibroma	0	0	1
	Cystadenoma	0	0	1
	Adenoma	14	14	15
Pituitary gland	Adenoma C-cell	2	4	3

Thyroid	**Adenocarcinoma**	0	2	0
Liver	Metastasis primary mesenchymal	1	1	3
In females				
Mammary gland	Fibroadenoma	7	8	14
	Multiple fibroadenoma	9	2	3
	Adenocarcinoma	3	0	2
Uterus	Adenoma			
	Adenonocarcinoma	0	0	1
	Myosarcoma	1	0	1
Thyroid	Adenoma C-cell	1	0	2
Pituitary gland	Adenoma	26	26	24
Liver	Meta. Primary mesenchymal	0	2	1

Malign tumours in bold

- *Local or multi-site responses:* no tumours
- *Progression of lesions to malignancy:* /
- *Gender and/or species-specific responses :* /
- *Tumour incidence data by sex, dose and tumour type:* /
- *Mode of action (genotoxic, non-genotoxic):* /
- *Toxic response data by sex and dose:* /
- *Tumour latency:* /

3.9.1.2 Animal data on NITROETHANE

3.9.1.2.1 Chronic inhalation toxicity study (Anonymous 35, 1986)

Study reference:

Anonymous 35, 1986

Detailed study summary and results:

Test type

- Similar to OECD TG 453
- GLP not specified
- Major deviations: The doses were not selected according the criteria of the guideline. Only 2 doses were tested. 40 animals/group. Some tissues were not examined microscospically (parathyroid, caecum, rectum, bone marrow, …).
- Reliability 2 (according to the registration dossier)

Test substance

- Nitroethane
- *Degree of purity:* 97.92 %

- *Impurities:* 0.01 % nitromethane, 2.07 % 2-nitropropane. As 2-nitropropane is classified Carc. Cat. 1B, the presence of this impurity above the generic concentration limit (0.1 %) would lead to a classification as Carc. Cat. 1B.

Test animals

- *Species/strain/sex:* rat / Long-Evans / both sexes
- *Nb. of animals per sex per dose:* 40 but due to an error during the study, 41 males and 39 females were used in the 200 ppm group.
- *Age and weight at the study initiation:* age not provided, weight : males : ~ 191 g, females : ~ 164 g.

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration of test/exposure period:* 2 y
- *Doses/concentration levels:* 0, 100 and 200 ppm. The rationale for dose level selection was not provided but were probably selected taking into account the occupational exposure limits as it is specified that these concentrations were "far above usual levels of human industrial exposure".
- *Frequency of treatment:* 7 hours/day, 5 days/week
- *Control group and treatment:* 1 control group + 2 doses
- *Historical control data:* not provided
- *Post exposure observation period:* no
- *Type of inhalation exposure and test conditions (e.g.: exposure apparatus):* vapours of nitroethane (NE) were generated by bubbling purified nitrogen through liquid NE in an all-glass vessel maintained in a thermostatted water bath at a temperature of 45 °C. Sufficient liquid NE to maintain a constant liquid level in the generator was added automatically.
- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* "whole body", exposure data : the concentration of NE within the chamber was monitored using a MIRAN 1A infra-red gas analyser.
- *Analytical verification of test atmosphere concentrations:* concentrations were monitored at least three and usually four times each day.

Results and discussion

- *Mortality and time to death:* no treatment-related effects

Table 52: Mortality rate

Concentration (ppm)	0	100	200
Male	20/40	21/40	16/41
Female	23/40	23/40	15/39

- *Clinical signs:* no effects

- *Body weight gain:*
  - *In males:* significantly lower than controls in males exposed to 100 ppm
  - *In females:* significantly lower than controls in females exposed to 200 ppm

  According to the authors, "the lack of well-defined dose-response relationship suggested the involvement of factors other than just exposure to nitroethane. Body weight may have been influenced by the fact that the control animals were not housed in an exposure chamber during the exposure periods".
- *Food/water consumption:* not detailed
- *Ophthalmoscopic examination:* no data
- *Clinical chemistry:* a slight but statistically significant increase of total protein and BUN was observed in females exposed to 200 ppm.
- *Haematology:* no statistically significant effects were observed. Htc levels were 37.4, 33.1 and 33.3 % (at 0, 100 and 200 ppm, respectively) and WBC levels were 13.1, 11.1 and 10.3 $x10^3$ (at 0, 100 and 200 ppm, respectively). The MetHb level was not reported.
- *Urinalysis:* no data
- *Organ weights:* no treatment-related effects (brain, liver, kidneys, lungs, heart)
- *Necropsy findings:* no effects reported
- *Histopathological findings:* "no other effects than usual age-associated degenerative diseases and endocrine target organ response to pituitary hyperplasia were observed and there were similar in controls and exposed animals."
- *Tumour incidence data by sex, dose and tumour type:* no treatment-related increase of tumours. In all animals, an increase in the incidence of benign tumours (adenoma of the pituitary gland) was observed but was similar in control and exposed animals.

  Maling tumours were very rare and were not treatment-related.

Table 53: Tumour incidence

Dose level exposure (ppm)			**0**	**100**	**200**
Pituitary Gland	Nodular hyperplasia	M (%)	13/38 (34)	15/39 (38)	15/40 (38)
		F (%)	7/38 (18)	6/40 (15)	12/37 (32)
	Adenoma	M (%)	22/38 (58)	16/39 (41)	16/40 (40)
		F (%)	27/38 (71)	26/40 (65)	23/37 (62)
	Nodular hyperplasia or adenoma	M (%)	35/38 (92)	31/39 (79)	31/40 (78)
		F (%)	34/38 (89)	32/40 (80)	35/37 (95)
Mammary gland	Adenoma	M (%)	0/30 (0)	0/29 (0)	0/27 (0)
		F (%)	0/39 (0)	1/39 (3)	0/36 (0)
	Fibroadenoma	M (%)	0/30 (0)	1/29 (3)	0/27 (0)

		F (%)	5/39 (13)	3/39 (8)	3/36 (8)
	Cystoadenoma	M (%)	2/30 (6)	0/29 (0)	1/27 (4)
		F (%)	2/39 (5)	2/39 (5)	1/36 (3)
	Multiple fibroadenoma	M (%)	0/30 (0)	0/29 (0)	0/27 (0)
		F (%)	0/39 (0)	3/39 (8)	1/36 (3)
	Adenocarcinoma	M (%)	0/30 (0)	0/29 (0)	0/27 (0)
		F (%)	1/39 (3)	0/39 (0)	0/36 (0)
Salivary gland	Adenoma	M (%)	0/39 (0)	1/40 (3)	0/39 (0)
		F (%)	0/40 (0)	0/40 (0)	0/39 (0)
	Undifferentiated carcinoma	M (%)	0/39 (0)	0/40 (0)	0/41 (0)
		F (%)	0/40 (0)	1/40 (3)	0/39 (0)
Brain	Astrocystome	M (%)	2/40 (5)	1/40 (3)	0/41 (0)
		F (%)	0/40 (0)	1/40 (3)	0/39 (0)
	Metastasis, primary mesenchymal:	M (%)	0/40 (0)	0/40 (0)	1/41 (3)
		F (%)	0/40 (0)	1/40 (3)	0/39 (0)
Liver	Hepatocarcinoma	M (%)	0/40 (0)	0/40 (0)	0/41 (0)
		F (%)	0/40 (0)	0/40 (0)	1/39 (3)
	Metastasis, primary epithelial	M (%)	0/40 (0)	0/40 (0)	0/41 (0)
		F (%)	0/40 (0)	4/40 (10)	0/39 (0)
	Metastasis, primary mesenchymal	M (%)	0/40 (0)	3/40 (8)	1/41 (3)
		F (%)	2/40 (5)	1/40 (3)	1/39 (3)
Spleen	Undifferentiated sarcoma	M (%)	0/40 (0)	0/40 (0)	0/41 (0)
		F (%)	0/40 (0)	0/40 (0)	1/39 (3)
	Metastasis, primary mesenchymal	M (%)	0/40 (0)	1/40 (3)	1/40 (3)
		F (%)	1/40 (3)	0/40 (0)	0/39 (0)
Kidney	Adenocarcinoma	M (%)	0/40 (0)	0/40 (0)	0/41 (0)
		F (%)	0/40 (0)	1/40 (3)	0/39 (0)
	Metastasis, primary epithelial	M (%)	0/40 (0)	0/40 (0)	0/41 (0)
		F (%)	0/40 (0)	3/40 (8)	0/39 (0)
	Angioma	M (%)	0/40 (0)	1/40 (3)	0/41 (0)
		F (%)	0/40 (0)	0/40 (0)	0/39 (0)
	Lipoma	M (%)	0/40 (0)	0/40 (0)	0/41 (0)
		F (%)	1/40 (3)	0/40 (0)	0/39 (0)
	Metastasis, primary mesenchymal	M (%)	0/40 (0)	1/40 (3)	0/41 (0)
		F (%)	2/40 (5)	1/40 (3)	0/39 (0)

Thymus	Undifferentiated sarcoma	M (%)	0/29 (0)	0/32 (0)	1/37 (3)
		F (%)	0/36 (0)	0/33 (0)	1/34 (3)
	Metastasis, primary mesenchymal	M (%)	0/29 (0)	1/32 (3)	1/37 (3)
		F (%)	1/36 (3)	0/33 (0)	0/34 (0)

An examination of the data in the tables indicated that the expected incidence of age-related degenerative diseases was seen in approximately equal frequencies in all groups, The incidence of nodular hyperplasia and adenomas of the pituitary gland associated with the endocrine target organ response was similar in all groups. These data do not indicate any significant hepatic pathologic difference between the control and exposed (NE) rat groups, but they do indicate a normal spontaneous risk of hepatic nodules in aged rats.

- *Local or multi-site responses:* no tumours
- *Progression of lesions to malignancy:* /
- *Gender and/or species-specific responses:* /
- *Tumour incidence data by sex, dose and tumour type:* /
- *Mode of action (genotoxic, non-genotoxic):* /
- *Toxic response data by sex and dose:* /
- *Tumour latency:* /

3.9.1.3 Animal data on 1-NITROPROPANE

3.9.1.3.1 Long term inhalation toxicity study (Griffin T.B. *et al.*, 1982)

Study reference:

Griffin T.B., Stein A.A. and Coulston F., 1982. Inhalation exposure of rats to vapors of 1-nitropropane at 100 ppm, Ecotox. Environ. Safety, 6, 268-282.

Detailed study summary and results:

Test type

- No guideline followed
- GLP compliance : not specified
- Reliability 2 (according to the registration dossier)
- 21.5 months inhalation study, 1 dose level + control.
- Groups of 10 males and 10 females were killed after 1 month, 3 months, 12 monhts and 18 months. Additional groups were removed from exposure after 3 and 12 months.
- Remaining animals were killed at the end of the study.
- All organs were examined at necropsy and sections from 26 organs and from any gross pathology were taken for microscopic examination but special care was given to the liver.

Test substance

- 1-nitropropane
- *Degree of purity:* not specified

Test animals

- *Species/strain/sex:* rat / Long-Evans / both sexes
- *Nb. of animals per sex per dose:* 125/sex

  Groups of rats (10/sex/group) were sacrificed after 1 m, 3 m, 12 m and 18 m of exposure

  Additional groups (10/sex/group) were removed from the exposure chamber after 3 m and 12 m and non exposed after that until the end of the exposure period

  All remaining animals alive were killed after 21.5 m
- *Age and weight at the study initiation:* not provided

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration of test/exposure period:* 21.5 m
- *Doses/concentration levels:* 0 and 100 ppm
- *Frequency of treatment:* 7 h/d, 5 d/week
- *Control group and treatment:* 1 control and 1 dose
- *Historical control data:* no data
- *Post exposure observation period:* groups of 10 females and 10 males were removed from exposure after 3 and 12 months and maintained under nonexposure conditions till the end of the study
- *Type of inhalation exposure and test conditions (e.g.: exposure apparatus):* vapours of 1-nitropropane were generated by bubbling purified nitrogen through liquid 1-Nitropropane in an all-glass vessel. The vapour of 1-Nitropropane and nitrogen were then introduced into the mixing chamber prior to their transfer to the exposure chamber. The vapour generator was maintained in a thermostatted water bath at a temperature of 45 °C.
- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* "whole body", exposure data : the concentration of 1-Nitropropane vapours was monitored by frequent sampling.
- *Analytical verification of test atmosphere concentrations:* Routinely at least 3 air samples were obtained daily.

Results and discussion

- *Mortality and time to death:* no details were given on the rate of mortality. The number of animals found dead was higher in exposed animals.
- *Clinical signs:* no details provided
- *Body weight gain: "*growth appeared normal for both sexes and only inconsistent differences were seen in body weights between control and exposed groups and likely due to the small sample size."

Table 54: Body weight data (in g)

	Males		Females	
Dose level (ppm)	0	100	0	100
1 m	381 (10)	367 (10)	247 (10)	219 (10)
3 m	509 (10)	484 (10)	300 (10)	288 (10)
12 m	655 (10)	580 (10)	341 (10)	333 (10)
18 m	674 (10)	651 (10)	428 (10)	349 (10)
21.5 m	671 (60)	629 (27)	397 (59)	413 (28)
3 m + 18.5 m of recovery	/	755 (4)	/	381 (4)
12 m + 9.5 m of recovery	/	636 (6)	/	357 (8)

() : number of animals examined

- *Food/water consumption:* not provided
- *Ophthalmoscopic examination:* no data
- *Clinical chemistry:* no effects
- *Haematology:* no effects

Table 55: Methemoglobin (in mg/dL)

	Males		Females	
Dose level (ppm)	0	100	0	100
1 m	25 (9)	32 (10)	13 (10)	29 (7)
3 m	24 (9)	30 (10)	38 (10)	49 (7)
12 m	16 (9)	22 (10)	17 (10)	22 (10)
18 m	36 (9)	49 (10)	36 (10)	29 (12[A])
21.5 m	120 (10)	70 (10)	74 (9)	46 (10)
3 m + 18.5 m of recovery	/	29 (4)	/	19 (3)
12 m + 9.5 m of recovery	/	43 (6)	/	50 (8)

() : number of animals examined ; [A] : DS's remarks : 12 animals noted in the full study report while 10 animals in the group

- *Urinalysis:* no data
- *Organ weights:* no effects on the weights of liver, kidney or brain.

Table 56: Liver weight data (in g)

	Males		Females	
	0 ppm	100 ppm	0 ppm	100 ppm
1 m	13.8 (10)	13.1 (10)	8.8 (10)	8.0 (10)
3 m	16.7 (10)	15.2 (10)	10.0 (10)	9.5 (10)
12 m	16.1 (10)	13.8 (10)	8.4 (10)	8.7 (10)

18 m	19.5 (10)	14.9 (10)	12.3 (10)	8.7 (10)
21.5 m	15.7 (60)	16.0 (27)	10.4 (59)	10.9 (28)
3 m + 18.5 m of recovery	/	16.7 (4)	/	10.0 (4)
12 m + 9.5 m of recovery	/	15.5 (6)	/	10.1 (8)

() : number of animals examined

Table 57: Kidney weight (in g)

	Males		Females	
Dose level (ppm)	0	100	0	100
1 m	2.86 (10)	2.84 (10)	1.90 (10)	1.74 (10)
3 m	3.57 (10)	3.38 (10)	2.05 (10)	2.05 (10)
12m	3.69 (10)	3.47 (10)	2.17 (10)	2.29 (10)
18m	4.87 (10)	4.00 (10)	2.68 (10)	2.47 (10)
21.5m	4.27 (59)	4.83 (24)	2.60 (58)	2.94 (27)
3m +18.5m of recovery	/	4.10 (4)	/	2.36 (4)
12m + 9.5m of recovery	/	3.78 (6)	/	2.50 (4)

() : number of animals examined

Table 58: Brain weight (in g)

	Males		Females	
Dose level (ppm)	0	100	0	100
1 m	2.01 (10)	2.01 (10)	1.85 (10)	1.86 (10)
3 m	2.12 (10)	2.17 (10)	1.95 (10)	1.95 (10)
12 m	2.27 (10)	2.25 (10)	2.15 (10)	2.23 (10)
18 m	2.25 (10)	2.23 (10)	2.07 (10)	2.02 (10)
21.5 m	2.21 (59)	2.27 (26)	1.97 (57)	2.00 (28)
3 m + 18.5 m of recovery	/	2.24 (4)	/	1.94 (4)
12 m + 9.5 m of recovery	/	2.25 (6)	/	1.93 (8)

() : number of animals examined

- *Necropsy findings:* no effects
- *Histopathological findings:* only few incidences of liver vacuolization and a number of parenchymal abscesses were found in dead or moribund animals.
- *Tumour incidence data by sex, dose and tumour type:*
    - *Benign tumours:*

Pituitary adenoma: an increase was observed after 18 months but without difference between controls and exposed animals

Table 59: Inc. of pituitary adenoma

	Tot. inc.	1 m		3 m		12 m		18 m	
		Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Tot.	94/406	0/14	0/15	0/17	0/16	1/13	1/15	9/19	5/49
M	18/205	0/6	0/8	0/10	0/8	0/8	1/7	2/10	2/10
F	76/201	0/8	0/7	0/7	0/8	1/5	0/8	7/9	3/9
	Tot. inc.	21.5 m		Animals found dead		Recovery period			
		Control	Exposed	Control	Exposed	3 m	12 m		
Tot.	94/406	34/112	9/49	14/39	10/45	6/17	5/16		
M	18/205	7/58	1/24	3/21	1/21	0/7	1/7		
F	76/201	27/54	8/25	11/18	9/24	6/10	4/9		

Islet adenoma: a slight increased was observed at the term of the study but without difference between controls and exposed animals.

Table 60: Inc of islet adenoma

	Tot. inc.	1 m		3 m		12 m		18 m	
		Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Tot.	14/485	0/20	0/20	0/20	0/20	0/20	0/20	0/19	0/19
M	13/240	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/9
F	1/245	0/10	0/10	0/10	0/10	0/10	0/10	0/9	0/10
	Tot. inc.	21.5 m		Animals found dead		Recovery period			
		Control	Exposed	Control	Exposed	3 m	12 m		
Tot.	14/485	7/118	6/52	0/47	0/72	0/19	0/19		
M	13/240	6/59	6/25	0/23	0/36	0/9	1/9		
F	1/245	1/59	0/27	0/24	0/36	0/10	0/10		

- *Malign tumours:*

The most common malignant tumour observed was lymphosarcoma in spleen and in lymph nodes after 18 months but the incidence in control and exposed animals was similar.

Table 61: Inc. of spleen lymphosarcoma

	Tot. inc.	1 m		3 m		12 m		18 m	
		Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Tot.	7/497	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20

M	3/249	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
F	4/248	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

	Tot. inc.	21.5 m		Animals found dead		Recovery period	
		Control	Exposed	Control	Exposed	3 m	12 m
Tot.	7/497	0/119	0/54	3/50	3/75	1/19	0/20
M	3/249	0/60	0/26	2/25	0/38	1/10	0/10
F	4/248	0/59	0/28	1/25	3/37	0/9	0/10

Table 62: Inc. of lymph nodes lymphosarcoma

	Tot. inc.	1 m		3 m		12 m		18 m	
		Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Tot.	6/469	0/20	0/20	0/19	0/19	0/19	0/20	0/20	0/20
M	3/232	0/10	0/10	0/9	0/9	0/9	0/10	0/10	0/10
F	3/237	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

	Tot. inc.	21.5 m		Animals found dead		Recovery period	
		Control	Exposed	Control	Exposed	3 m	12 m
Tot.	6/469	0/111	1/51	3/47	1/66	1/19	0/18
M	3/232	0/55	0/26	2/22	0/33	1/10	0/9
F	3/237	0/56	1/25	1/25	1/33	0/9	0/9

3.9.1.3.2 Assay of 1-nitropropane, 2-nitropropane, 1-azoxypropane and 2-azoxypropane for carcinogenicity (Fiala E.S. *et al.*, 1987 (as reported in MAK Value Documentation, 1999 & 2017))

Study reference:

Fiala E.S., Czerniak R., Castonguay A., Conaway C.C., Rivenson A., 1987. Assay of 1-nitropropane, 2-nitropropane, 1-azoxypropane and 2-azoxypropane for carcinogenicity by gavage in Sprague-Dawley rats, Carcinogenesis, 8, 1947-1949.

Detailed study summary and results:

Test type

- 1-Nitropropane was administered by gavage 3 times/week for 16 weeks, followed by 1 time/week for 10 weeks. Surviving animals (26) were sacrified after 77 weeks.
- No guideline followed
- Not GLP
- Reliability 2 (according to the registration dossier, however only summary available)

Test substance

- 1-nitropropane

- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* Rat / SD / male
- *Nb. of animals per sex per dose:* not specified
- *Age and weight at the study initiation:* 150-160 g

Administration/exposure

- *Route of administration:* oral (gavage)
- *Duration of test/exposure period:* 26 w (16 w + 10 w)
- *Doses/concentration levels:* 0 and 89.1 mg/kg bw/d
- *Frequency of treatment:* 3 times / week for 16 weeks, followed by 1 time/week for 10 weeks
- *Control group and treatment:* 1 control and 1 dose
- *Historical control data:* no
- *Post exposure observation period:* 51 w (surviving animals were sacrified after 77 w)
- *Vehicle:* 10 % aqueous Emulphor EL-620

Results and discussion

- *Mortality and time to death:* not specified
- *Clinical signs:* not specified
- *Body weight gain:* treatment related effects observed
- *Food/water consumption:* not specified
- *Ophthalmoscopic examination:* not examined
- *Clinical chemistry:* not examined
- *Haematology:* not examined
- *Urinalysis:* not examined
- *Organ weights:* not specified
- *Necropsy findings:* treatment related effects observed
- *Histopathological findings:* no effects observed
- *Tumour incidence data by sex, dose and tumour type:* no increase of tumour incidence (no more details given)

3.9.1.3.3 Tests for chemical carcinogens (Hadidian Z. *et al.*, 1968 (as reported in MAK Value Documentation, 1999 & 2017))

Study reference:

Hadidian Z, Fredrickson N, Weisburger EK, Weisburger JH, Glass RM, Mantel N, 1968. Tests for chemical carcinogens. Report on the activity of derivatives of aromatic amines, nitrosamines, quinolines, nitroalkanes, amides, epoxides, aziridines, and purine antimetabolites, J Natl Cancer Inst, 41, 985-1036.

Detailed study summary and results:

Test type

- 1-Nitropropane was administered by gavage 5 times/week for 52 weeks. In the first study, 3 doses were tested. In the second study, only one dose was tested.
- No guideline followed
- Not GLP compliant.
- Not reported in the registration dossier

Test substance

- 1-nitropropane
- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* Rat / F344 / both sexes
- *Nb. of animals per sex per dose:* 3/sex/group (except for the mid dose: 15/sex)
- *Age and weight at the study initiation:* not reported

Administration/exposure

- *Route of administration:* oral (gavage)
- *Duration of test/exposure period:* 52 w
- *Doses/concentration levels:* 0, 0.3, 3 or 10 mg/day
- *Frequency of treatment:* 5 d/w
- *Historical control data:* no data
- *Post exposure observation period:* no
- *Vehicle:* no data

Results and discussion

- *Tumour incidence data by sex, dose and tumour type:* No increase of tumour incidence.
- No more details given

3.9.2 Human data

No human data available

3.9.3 *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

See chapter 3.8

3.9.4 Other data (e.g. studies on mechanism of action)

No other data available

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Animal data on NITROMETHANE

3.10.1.1.1 Prenatal developmental toxicity study in rats (Anonymous 36, 2017)

Study reference:

Anonymous 36, 2017

Detailed study summary and results:

Test type

- According to OECD TG 414
- GLP-compliant
- Reported deviations: identification of males via a subcutaneous transponder and not a mark on the tail, variation of the relative humidity from 44.9 to 65 % and no use of the surplus animals for training purpose.
- Reliability 1 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* > 99 %
- *Impurities: /*

Test animals

- *Species/strain/sex:* rat / Wistar / females and untreated males
- *Nb. of animals per sex per dose:* 24 females in each group. 104 females mated with 52 males to yield 24 mated females per group
- *Age and weight at the study initiation: /*

Administration/exposure

- *Route of administration:* inhalation
- *Duration and frequency of test/exposure period:* daily exposure during 6h, from GD 6 to 20
- *Doses/concentration levels:* 0, 300, 600 and 1200 ppm
- *Control group and treatment:* yes
- *Vehicle:* air
- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* whole body
- *Analytical verification of test atmosphere concentrations:* yes

- *Particle size:* not applicable

Description of test design:

- *Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):* 2 females: 1 male per cage to obtain 24 mated females per dose group, pregnancy was determined according to the presence of sperm in vaginal smears (performed daily, if sperm was notified, then GD 0 was determined)
- *Premating exposure period for males and females:* /
- *Standardization of litters (yes/no and if yes, how and when):* /
- *Parameters assessed for P and F1:* in all animals, the nose, larynx, trachea, lungs, liver, sciatic nerve and gross lesions were removed and imerged in formalin for possible histopathological examination. From P females killed on GD 21, the number of corpora lutea, implantation sites, early/late resorption, live/dead foetuses, gross malformed foetuses, the sex of foetuses, the gross assessment of the placenta and abnormal tissues in P females were examined. Some organs were weighed (kidneys, liver, uterus, uterus+placenta+foetuses, live foetuses and placenta. The sex ratio, gestation index, pre- and post-implantation losses were calculated.
  In foetuses, the gross abnormalities were observed. Half of the litters were examined for soft tissues abnormalities, the other half was assessed for skeletal anomalies.
- *Clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):* mortality, pre-exposure signs on the skin, and during-dosing anomalies

Results and discussion

- *Actual dose received by dose level by sex if known:*

Table 63: Actual dose

Target dose (ppm)	300	600	1200
Actual dose	303	601	1178
Standard Deviation	3.3	12.4	43.9

For P adults (per dose):

- *Time of death during the study and whether animals survived to termination:* No mortality observed in any dose group
- *Clinical observations:* no data
- *Body weight data for P animals:*

Table 64: BW at the start of the study in females and evolution during gestation

Dose (ppm)	0	300	600	1200
Nb	17	20	20	22
GD 0	207.71 ± 11.32	213.26 ± 10.32	208.86 ± 10.67	210.99 ± 8.80

GD 6	234.05 ± 11.73	239.10 ± 13.05	236.06 ± 12.63	237.24 ± 12.16
GD 9	240.90 ± 12.2	247.87 ± 14.26	243.16 ± 12.39	240.70 ± 12.11
GD 12	252.52 ± 13.78	261.27 ± 14.42	254.01 ± 15.14	251.51 ± 13.61
GD 15	264.63 ± 14.36	273.01 ± 14.60	266.45 ± 14.98	265.07 ± 13.46
GD 18	293.29 ± 17.03	303.72 ± 17.68	294.13 ± 17.54	279.79* ± 15.84
GD 21 (termination)	329.28 ± 22.15	338.91 ± 21.18	326.43 ± 21.99	287.24** ± 24.97

Table 65: BW modifications in females, during gestation

Dose (ppm)	0	300	600	1200
Nb	17	20	20	22
GD 0-6	26.35 ± 3.22	25.85 ± 6.37	27.20 ± 6.13	26.25 ± 5.72
GD 6-9	6.85 ± 2.42	8.77 ± 3.39	7.10 ± 2.37	3.46** ± 3.11
GD 9-12	11.62 ± 3.29	13.40 ± 2.90	10.86 ± 6.63	10.81 ± 3.37
GD 12-15	12.11 ± 2.68	11.74 ± 3.55	12.43 ± 6.57	13.56 ± 4.10
GD 15-18	28.66 ± 5.08	30.71 ± 5.78	27.68 ± 4.05	14.72** ± 10.33
GD 18-21	35.98 ± 7.19	35.20 ± 5.94	32.30 ± 5.75	7.45** ± 15.27
GD 0-21	121.57 ± 15.06	125.66 ± 16.37	117.57 ± 15.05	76.25** ± 24.20

- *Food consumption:*

Table 66: Food consumption in females

Dose (ppm)	0	300	600	1200
Nb	17	20	20	22
GD 0-6	17.81 ± 1.54	18.23 ± 1.79	17.57 ± 1.64	17.79 ± 2.26
GD 6-9	19.02 ± 1.69	18.88 ± 1.88	17.78 ± 1.79	15.93** ± 2.40
GD 9-12	19.57 ± 1.43	20.90 ± 3.97	19.86 ± 3.14	18.45 ± 2.27
GD 12-15	19.95 ± 2.80	20.56 ± 2.40	20.47 ± 2.83	19.54 ± 1.96
GD 15-18	21.40 ± 2.29	22.17 ± 2.81	21.51 ± 3.49	20.35 ± 2.61
GD 18-21	19.84 ± 2.07	20.98 ± 1.79	20.38 ± 2.35	17.66* ± 2.04

- *Toxic response data by sex and dose including indices of mating, fertility, gestation, birth, viability and lactation; indicate the numbers used in calculating the indices:* /
- *Haematological and clinical biochemistry findings:* no data
- *Effects on sperm:* not assessed
- *Number of P females cycling normally and cycle length:* /
- *Duration of gestation (calculated from day 0 of pregnancy):* 21 d, ceasarian section
- *Reproduction parameters:*

Table 67: Reproductive parameters

Dose (ppm)	0	300	600	1200
Nb	17	19	20	22
Corpora lutea/dam	14.1	14.2	12.9	13.6
Implantation sites/dam	12.2	12.2	11.6	12.6
% pre-impl. loss/dam	12.5	13.6	10.4	8.2
Mean nb early resorptions/dam	0.2	0.2	0.4	0.4
% early resorptions/dam	1.3	1.2	3.5	3.3
Mean nb late resorptions/dam	0.1	0.1	0.1	6.5**
% late resorptions/dam	0.9	0.4	0.4	50.5**
Mean nb post-impl. loss/dam	0.3	0.3	0.5	6.9**
% post-impl. loss/dam	2.2	2.1	3.9	53.8**
Mean nb foetuses/animal	11.9	12.0	11.2	5.7**
% live foetuses	100	99.6	100	100
Nb dead foetuses	0	1	0	0
Mean nb live foetuses/animal	11.9	11.9	11.2	5.7**
Nb malformed (external)	0	0	0	1
Sex ratio (% males)	48.2	42.0	51.5	44.8

- *Data on functional observations: /*
- *Necropsy findings*

Table 68: Gross evaluation in dams

Doses (ppm)	0	300	600	1200
Nb females evaluated	24	24	24	24
Nb macro. lesions	21	18	20	13
Hemorrhagic vaginal fluid	-	-	-	8
Swollen uterus	3	1	2	1
Pale liver	0	1	0	1

- *Body weight at sacrifice and absolute and relative organ weight data for the parental animals:*

Table 69: Organ weights in females

Dose (ppm)	0	300	600	1200
Terminal BW (D 21)	329.28 ± 22.15	337.51 ± 20.77	326.41 ± 22.04	287.24** ± 24.97
Gravid uterus (g)	76.730 ± 13.817	80.029 ± 14.080	72.779 ± 11.464	35.764** ± 21.653
Empty uterus (g)	4.7554 ± 0.8585	4.9136 ± 0.8269	4.6620 ± 0.5930	3.7435 ± 0.5496
Ovaries (absolute) (g)	0.1186 ±0.0129	0.1283 ± 0.0117	0.1223 ± 0.0140	0.1202 ± 0.0216

Ovaries (relative) (%)	0.0360 ± 0.0036	0.0381 ± 0.0034	0.0375 ± 0.0037	0.0420** ± 0.0071
Placenta (g)	0.44 ± 0.04	0.46 ± 0.05	0.47 ± 0.02	0.42 ± 0.04
Liver (absolute) (g)	10.7228 ± 0.9706	11.3909 ± 0.8206	10.9018 ± 0.9298	11.3716 ± 1.0548
Liver (relative) (%)	3.2572 ± 0.2065	3.3789 ± 0.2048	3.3632 ± 0.3029	3.9670** ± 0.2843
Kidneys (absolute) (g)	1.3716 ± 0.1276	1.4724* ± 0.1175	1.4840* ± 0.1179	1.6044** ± 1.1222
Kidneys (relative) (%)	0.4175 ± 0.0384	0.4366 ± 0.0276	0.4576 ± 0.0357	0.5623** ± 0.0631

- *Histopathological findings:* /
- *Body weight change and gravid uterine weight, including optionally, body weight change corrected for gravid uterine weight:* /

For F1 pups/litters (per dose):

- *Mean number of live pups (litter size):* 11.9, 11.9, 11.2 and 5.7**, resp. at 0, 300, 600 and 1200 ppm
- *Sex ratio:* 48.2, 42.0, 51.5 and 44.8 % of males, resp. at 0, 300, 600 and 1200 ppm
- *Viability index (pups surviving 4 days/total births):* /
- *Survival index at weaning:* /
- *Mean litter or pup weight by sex and with sexes combined:*

Table 70: Foetuses BW

Doses (ppm)	0	300	600	1200
Nb of females	17	19	20	17
Female bw	4.80 ± 0.31	4.91 ± 0.25	4.76 ± 0.34	3.65** ± 0.37
Nb of males	16	18	20	17
Male bw	4.96 ± 0.25	5.10 ± 0.15	4.98 ± 0.34	3.93** ± 0.42

- *External, soft tissue and skeletal malformations and other relevant alterations:* Subcutaneous edema, listed as external malformation, was seen on one foetus from the high dose group. Regarding variations, subcutaneous hemorrhages was reported on two foetuses, one in the control group and one in the high dose group. Furthermore, in the high dose group, a significant increase in the number of pale foetuses (13/17 litters) was recorded.

  No effects were seen in the low and middle dose groups.

  - *External examination:*

Table 71: Effects on foetuses (external malformations and variations)

Doses (ppm)	0	300	600	1200
Nb foetuses examined	202	227	223	126
Nb litters examined	17	19	20	17
Nb foetuses with Malformations (Nb litters affected)	2 (2/17)	0 (0/19)	1 (1/20)	10 (5/17)
% foetuses malformed/litter	1.2	0.0	0.4	8.4

Nb External malf. (%/litter)	0 (0)	0 (0)	0 (0)	1 (0.05)
Nb foetuses with Subcutaneous edema (%/litter)	0 (0)	0 (0)	0 (0)	1 (0.05)
Nb foetuses with Variations (Nb litters affected)	141 (17/17)	140 (19/19)	146 (20/20)	121 (17/17)
% foetuses with variation/litter	68.9	62.0	64.6	94.4**
Nb ext. variations (%/litter)	1 (0.5)	0 (0.0)	0 (0.0)	105 (76.52**)
Nb litters affected with ext. variations	1	0	0	13**
Nb foetuses with Subcutaneous haemorrhage (%/litter)	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.8)
Nb Pale foetuses (%/litter)	0 (0.0)	0 (0.0)	0 (0.0)	105 (76.5**)

- *Visceral examination:* No visceral malformations were observed in any group.

Table 72: Visceral variations in foetuses

Doses (ppm)		**0**	**300**	**600**	**1200**
Nb foetuses		97	108	105	57
Nb litters		17	19	20	17
Nb affected foetuses (%/litter)		38 (37.7)	22 (21.0)	32 (29.6)	9 (18.8*)
Eyes Nb foetuses affected (%/litter)	-	7 (8.0)	6 (5.5)	18 (17.1)	7 (16.9)
	% litters affected	41.2	26.3	50.0	29.4
	Retina,fold, uni	5 (5.9)	5 (4.4)	17 (16.3)	7 (16.9)
	Retina, fold	2 (2.0)	1 (1.1)	1 (0.8)	0 (0.0)
Ureters Nb foetuses affected (%/litter)	% litters affected	41.2	36.8	10.0	5.9
	-	11 (11.2)	11 (10.9)	2 (1.7)	1 (1.0*)
	Bent	0	2 (2.1)	0	0

- *Skeletal examination:*

Table 73: Skeletal malformations

Doses (ppm)	**0**	**300**	**600**	**1200**
Nb foetuses examined	105	119	118	69
Total skel. Obs. Nb (%/litter)	2 (2.2)	0 (0.0)	1 (0.7)	10 (16.4)
% litters affected	2	0	1	5
Sternebra [Nb (%/litter)]	0 (0.0)	0 (0.0)	0 (0.0)	9 (10.5**)
Fused sternebra [Nb (%/litter)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)
Split sternebra [Nb (%/litter)]	0 (0.0)	0 (0.0)	0 (0.0)	8 (9.7*)

Table 74: Skeletal variations

Doses (ppm)	0	300	600	1200
Nb foetuses examined	105	119	118	69
Total skel. Obs. Nb (%/litter)	103 (97.1)	118 (99.1)	114 (95.8)	69 (100)
Pubis - Incomplete ossification [Nb (%/litter)]	0 (0.0)	0 (0.0)	0 (0.0)	6 (14.2*)
Forelimbs, phalanges front proximal [Nb (%/litter)]	62 (57.8)	63 (53.8)	62 (52.3)	64 (89.7*)
1-4 unossified digits [Nb (%/litter)]	23 (21.0)	23 (20.1)	25 (20.5)	49 (65.6**)
Sternebra [Nb (%/litter)]	58 (56.3)	69 (57.4)	66 (55.8)	61 (90.5**)
Incomplete ossification of 3 or more sternebrae [Nb (%/litter)]	1 (1.0)	2 (2.8)	5 (4.2)	17 (21.0*)
Bipartite ossification [Nb (%/litter)]	1 (0.8)	0 (0.0)	0 (0.0)	12 (22.1**)
Ribs [Nb (%/litter)]	1 (1.0)	3 (2.6)	18 (14.7*)	34 (47.3**)
2 or more wavy ribs [Nb (%/litter)]	0 (0.0)	3 (2.6)	15 (12.5)	34 (47.3**)
Hindlimbs metatarsal [Nb (%/litter)]	26 (23.1)	20 (17.0)	44 (36.8)	55 (74.9**)
1-2 unossified metatarsals [Nb (%/litter)]	7 (6.4)	5 (4.9)	9 (7.4)	51 (69.8**)
Incomplete ossification interparietal skull [Nb (%/litter)]	1 (1.0)	3 (2.4)	3 (3.3)	16 (24.9**)
Bent ulna [Nb (%/litter)]	0 (0.0)	0 (0.0)	0 (0.0)	8 (20.1**)
Vertebra cervical bodies [Nb (%/litter)]	10 (8.9)	6 (5.9)	6 (5.0)	48 (67.7**)

- *Data on physical landmarks in pups and other postnatal developmental data:* /
- *Data on functional observations:* /

3.10.1.1.2 Reproductive toxicity study in rats (Whitman *et al.*, 1977)

Study reference:

Whitman R., Maher B. and Abeles R., 1977. Deficits in discrimination and maze learning resulting from maternal histidinemia in rats, J Abnorm Psychol, 86(6), 659-661.

Detailed study summary and results: rats received ip injection of nitromethane during one week (once every 3 days) before mating, then during mating and pregnancy. After one week of exposure, two males/group were introduced until dams were feconded. Histidine levels in dams plasma were kept high either with nitromethane injection, or with a high-histidine diet. The histine level in urine was followed during gestation. The pups remained with their mother until weaning, then they were exposed to a normal diet until they were 2-month old. The behaviour of the F1 was assessed 2 months and a half after birth. Results showed an impaired learning activity in all groups, but it was greater in high-histidine diet groups than in nitromethane exposed groups.

Test type

- Not following guidelines
- Disregarded study because of unsufficient reporting
- Reliability 4 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* rat/ albino
- *Nb. of animals per sex per dose:* unknown
- *Age and weight at the study initiation:* unknown

Administration/exposure

- *Route of administration:* IP injection
- *Duration and frequency of test/exposure period:* exposure one week prior to mating, then during mating and pregnancy; injection once every 3 days
- *Doses/concentration levels:* 0.5 mL of 1.5 M nitromethane
- *Control group and treatment:* yes only saline injection and high-histidine diet
- *Historical control data if available:* /
- *Vehicle:* physiological saline
- *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* 1.5 M nitromethane in 0.9 % NaCl

Description of test design:

- *Premating exposure period for males and females:* treatment began 1 week prior to mating
- *Dosing schedules and pre and post dosing observation periods:* 4 groups were determined: one receiving normal amount of histidine in diet and saline ip injections once every third day (control group), one receiving a high-histidine diet and saline ip injections, one receiving normal amount of

histidine in diet and nitromethane ip injection and a fourth group with high-histidine diet and nitromethane ip injections.

Results and discussion

- Equivalent number of pregnant dams per group
- Equivalent number of pups per litter per group
- Low death rates in pups of all dose groups
- Similar birth weights in all groups
- Lower BWG in pups feeding with histidine diets
- Significant impaired learning was evidenced in all treated groups, in comparison with the control group. The effects were greater in rats exposed to histidine than nitromethane.

For P adults (per dose):

- *Number of animals at the start of the test and matings:* 2 males per group, unknown number of females

No effects observed on fetotoxicity. All treated groups had impaired maze learning compared to controls.

3.10.1.2 Animal data on NITROETHANE

3.10.1.2.1 Teratology study in mice subjected to inhalation of diethylhydroxylamine, nitroethane and diethylamine hydrogen sulphite (Heicklen *et al.*, 1979)

Study reference:

Heicklen *et al.*, 1979. Teratology study in mice subjected to inhalation of diethylhydroxylamine, nitroethane and diethylamine hydrogen sulphite, Environ. Res., 20, 450-454.

Detailed study summary and results: three-generation reproductive toxicity

Test type

- Prior to GLP and guidelines
- Co-exposure to 3 chemicals, with low concentration of nitroethane
- Disregarded

3.10.1.2.2 Teratology study in mice subjected to inhalation of diethylhydroxylamine, nitroethane and diethylamine hydrogen sulphite (Beliles *et al.*, 1978)

Study reference:

Beliles *et al.*, 1978. Teratology study in mice subjected to inhalation of diethylhydroxylamine, nitroethane and diethylamine hydrogen sulphite, Environ. Res., 17, 165-176.

Test type

- Prior to GLP and guidelines
- Co-exposure to 3 chemicals, with low concentration of nitroethane

- Disregarded

3.10.1.3 Animal data on 1-NITROPROPANE

3.10.1.3.1 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (Anonymous 37, 2003)

Study reference:

Anonymous 37, 2003

Detailed study summary and results:

Test type

- Screening for reproductive and developmental toxicity according to OECD TG 422 with no deviations reported
- GLP
- Reliability 1 (according to the registration dossier)

Test substance

- 1-nitropropane
- *Degree of purity:* 99.69 %

Test animals

- *Species/strain/sex:* Rat / SD / both sexes
- *Nb. of animals per sex per dose:* 12
- *Age and weight at the study initiation:* aged 8 weeks at study initiation

Administration/exposure

- *Route of administration:* inhalation (vapours)
- *Duration and frequency of test/exposure period:*
  - *females:* 14 d prior mating, during mating (2 weeks) and until gestation day 19
  - *males:* 14 d prior mating and during mating, for a minimum of 28 d
- *Doses/concentration levels:* 0, 25, 50 and 100 ppm (= nominal concentrations) equivalent to 0, 24, 48 and 96 ppm (actual average concentrations in chamber) (corresponding to approx. 0, 0.092, 0.184 and 0.369 mg/L).
- *Historical control data:* yes
- *Vehicle:* air
- *Type of inhalation exposure and test conditions (e.g.: exposure apparatus):* vapours
- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* whole-body, exposure chamber
- *Analytical verification of test atmosphere concentrations:* yes, 0, 24, 48 and 96 ppm (actual mean chamber concentrations)

Description of test design:

- *Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):* 1:1 mating until pregnancy occurred or max 2 weeks. Mating was determined through daily vaginal lavage samples with presence of sperm assessment. If presence of sperm or vaginal plug observed, GD 0 was claimed. Afterwards, females were separated from males.
- *Premating exposure period for males and females (P and F1):* 14 d
- *Dosing schedules and pre and post dosing observation periods for P and F1:* dams were daily exposed to the test substance 2 weeks before mating, through mating (2weeks) to gestation day 19. Necropsy was performed on post-natal day (PND) 5. Parental males were dosed 2 weeks prior to mating, through mating (2 weeks) until test day 29 where they were necropsied.
- *Standardization of litters (yes/no and if yes, how and when):* /
- *Parameters assessed for P and F1:* effects on the reproductive and neurological systems were assessed as well as general toxicity. Gross necropsy of the parental generation was performed and organs were removed and weighed and extensive histological analysis was achieved.
- *Estrous cycle length and pattern, sperm examination, clinical observations performed and frequency:* no data on oestrous cyclicity, for sperm: spermatogenesis stages were assessed qualitatively, an histopathological examination of the testes was performed.
- *Parameters assessed for F1:* parturition date, litter size on the day of parturition (LD 0), number of live and dead pups on PND 0, 1, and 4, and pups sex and bodyweight on PND 1 and 4. Physical abnormalities or changes in the neonates were noted during the lactation period. If death occurred prior to the end of the study, pups were examined for external and visceral anomalies and sexed. All surviving pups were euthanized at PND 4 and examined histopathologically (only testes, nose/pharynx and gross abnormalities).
- *Post exposure observation period:* females were necropsied on post-partum day 5. Males were necropsied at the end of the exposure period (day 29)

Results and discussion

- *Actual dose received by dose level by sex if known:* mean chamber concentrations were 0, 24.4 ± 1.8, 48.4 ± 1.8, and 96.3 ± 2.6 ppm when targeting 0, 25, 50 and 100 ppm, respectively.

For P adults (per dose):

- *Time of death during the study and whether animals survived to termination:* no effects, no mortality was observed during the study
- *Clinical observations:* no effects
- *Body weight data for P animals:* body weights in males slightly decreased at the highest dose and significantly reduced at test day 7 (premating period), however a trend to decrease was observed at all observation times at the highest dose level. No changes were observed in females body weights. Consumption was decreased at the highest dose during the first week of the pre-mating period in

both sexes, in comparison with the control group. Then no difference was observed in treated and control groups.

Table 75: Male body weights summary (in g)

Day	0 ppm	25 ppm	50 ppm	100 ppm
-4	263.6 ± 11.5	263.4 ± 11.5	263.9 ± 11.4	263.9 ± 11.2
1	288.8 ± 14.3	287.6 ± 16.0	290.0 ± 14.6	382.8 ± 12.4
7	317.0 ± 19.5	315.0 ± 19.2	319.1 ± 21.6	295.0* ± 19.8
14	344.7 ± 23.9	344.4 ± 20.8	348.6 ± 27.5	323.1 ± 18.7
22	367.5 ± 28.0	373.1 ± 26.5	374.3 ± 31.0	345.1 ± 18.4
28	390.6 ± 25.7	393.5 ± 28.2	395.6 ± 32.1	368.8 ± 18.9

Table 76: body weight data (in g) in females

Dose level (in ppm)		0	25	50	100
Nb		12	12	12 in premating 10 starting from GD 7	
Premating period	D 1	215.9	218.2	215.5	216.5
	D 7	226.4	228.3	226.2	220.8
	D 14	235.5	240.7	241.7	235.3
Gestation period	D 7	273.1	282.3	276.6	272.5
	D 20	375.4	386.2	388.0	372.5
Lactation period	D 1	277.3	287.6	290.7	292.3
	D 4	296.5	306.9	309.6	305.8

- *Toxic response data by sex and dose including indices of mating, fertility, gestation, birth, viability and lactation:* At the mid and high dose levels, 2 females failed to be pregnant (fertility index of 100, 100, 83.3 and 83.3 % resp. at 0, 25, 50 and 100 ppm; HCD : range between 2000 to 2004 : 83.3 – 100 %).
    - *Mating index:* 100 % for both sexes.
    - *Gestation index:* 100 %.

Table 77: Fertility index (FI)

Exposure level (ppm)	0	25	50	100
M/F FI (%)	100	100	83.3 (10/12)	83.3 (10/12)
Study # & year	1-2000	2-2003	3-2004	4-2004
HCD FI (%)	100	91.7	91.7	83.3

- *Haematological and clinical biochemistry findings:*
    - *Hematological examination*: no statistically significant changes

      Methemoglobin : 1.7, 1.6, 1.6 and 1.5 % in males and 1.0, 1.0, 1.5 and 1.0 % in females, resp. at 0, 25, 50 and 100 ppm
    - *Clinical biochemistry evaluation*: only one significant change was observed in males (albumin : 3.2, 3.3, 3.3 and 3.4* g/dL resp. at 0, 25, 50 and 100 ppm)
- *Effects on sperm:* no effects observed
- *Reproduction data:* Mating index were of 100 % in all dose in both sexes. However, at the mid and high dose levels, 2 females failed to be pregnant (fertility index of 100, 100, 83.3 and 83.3 % respectively at 0, 25, 50 and 100 ppm; HCD : range between 2000 to 2004 : 83.3 – 100 %).
- *Number of P females cycling normally and cycle length:* /
- *Duration of gestation (calculated from day 0 of pregnancy):* 21.3, 21.5, 21.4 and 21.8 d resp. at 0, 25, 50 and 100 ppm

Table 78: Duration of gestation

Exposure level (ppm)	0	25	50	100
Duration in days	21.3 ± 0.5	21.5 ± 0.5	21.4 ± 0.5	21.8 ± 0.4

- *Precoital interval (number of days until mating and number of estrous periods until mating):*

Table 79: Time to mating

Exposure level (ppm)	0	25	50	100
In days	2.9 ± 1.2	3.6 ± 3.2	2.8 ± 3.6	3.5 ± 2.4

- *Number of implantations, corpora lutea, litter size:*

Table 80: Litter size

Dose	0	25	50	100
Born live	14.0 ± 1.8	14.3 ± 2.1	15.1 ± 1.7	11.9 ± 4.3
Born dead	0.2 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3

- *Number of pre- and post-implantation loss:*
    - *% of post-implantation loss :* 5.43, 7.98, 3.97 and 7.06 % resp. at 0, 25, 50 and 100 ppm

Table 81: Post-implantation loss

Exposure level (ppm)	0	25	50	100
Post-implantation loss (%)	5.43 ± 7.04	7.98 ± 7.64	3.97 ± 4.65	7.06 ± 10.71

- *Data on functional observations:* /

- *Necropsy findings:* no treatment-related findings
- *Body weight at sacrifice and absolute and relative organ weight data for the parental animals:* no statistically significant changes.

Table 82: Organ weight data (in g and g/100)

		Males				Females			
Dose level (in ppm)		0	25	50	100	0	25	50	100
FBW		354.1	358.8	357.3	328.7*	257.8	264.0	268.1	271.9
Adrenal glands	Abs	0.075	0.074	0.075	0.065	0.094	0.093	0.090	0.085
	Rel	0.021	0.021	0.021	0.020	0.037	0.035	0.034	0.031
Brain	Abs	1.986	2.024	2.035	2.040	1.917	1.985	1.970	1.952
	Rel	0.562	0.567	0.572	0.622*	0.747	0.755	0.738	0.720
Heart	Abs	1.161	1.204	1.241	1.157	0.913	0.961	0.986	1.022
	Rel	0.328	0.335	0.348	0.352	0.355	0.364	0.369	0.376
Kidneys	Abs	2.573	2.676	2.676	2.392	1.880	1.979	2.074	1.973
	Rel	0.726	0.747	0.749	0.729	0.730	0.749	0.776	0.724
Liver	Abs	10.108	10.641	10.627	9.310	9.230	9.887	10.028	10.340
	Rel	2.846	2.968	2.965	2.833	3.581	3.746	3.748	3.785
Spleen	Abs	0.605	0.620	0.622	0.619	0.609	0.581	0.581	0.609
	Rel	0.171	0.172	0.174	0.187	0.237	0.221	0.216	0.224
Thymus	Abs	0.381	0.317*	0.388	0.343	0.199	0.193	0.250	0.220
	Rel	0.107	0.088*	0.109	0.104	0.077	0.072	0.093	0.081
Thyroid	Abs	0.0177	0.0186	0.0199	0.0165	0.0147	0.0143	0.0159	0.0151
	Rel	0.0050	0.0052	0.0055	0.0050	0.0057	0.0054	0.0059	0.0056
Epididymides	Abs	1.024	1.070	1.038	1.054	-	-	-	-
	Rel	0.290	0.299	0.291	0.322	-	-	-	-
Testes/Ovaries	Abs	3.066	3.230	3.015	3.162	0.132	0.140	0.127	0.132
	Rel	0.867	0.902	0.846	0.965*	0.051	0.053	0.048	0.049

- *Histopathological findings:* effects were observed in the nasal tissue at mid dose in females and at the high dose in both sexes (such as multifocal degeneration of the olfactory epithelium, sometimes with signs of inflammation).

Table 83: Incidence of nasal tissue degeneration

Sex	Males				Females			
Dose level (in ppm)	0	25	50	100	0	25	50	100
Nb of animal examined	12	12	12	12	12	12	12	12

Within normal limits		12	12	12	9	9	10	8	1
Degeneration of the O.E. (multifocal)	VS	0	0	0	1	0	0	0	5
	S	0	0	0	1	0	0	0	2
Degeneration of the O.E. with inflammation (focal)	VS	0	0	0	0	0	0	2	0
Degeneration of the O.E. with inflammation (multifocal)	S	0	0	0	0	0	0	0	2
Chronic inflammation of the E. (squamous cell) (focal)	VS	0	0	0	0	2	1	0	0
	S	0	0	0	0	0	0	0	1
Chronic inflammation of the E. (squamous cell) (multifocal)	VS	0	0	0	0	1	1	1	2
	S	0	0	0	1	0	0	2	1

O.E.: olfactory epithelium ; E.: epithelium; V.S.: very slight, S: slight

For F1 pups/litters (per dose):

- *Mean number of live pups (litter size):*

Table 84: Live births

Exposure level (ppm)	0	25	50	100	HCD Study # & year	1-2000	2-2003	3-2004	4-2004
Mean nb of live pups at birth	14.0	14.3	15.1	11.9	# born live pups	13.6	15.1	15.6	13.3
Mean nb of live pups at D 1	13.8	14.3	15.1	11.8	Live pups D1	13.4	15.1	15.5	12.8
Live pups at D 4	13.8	14.1	15.1	11.8	Live pups D4	13.4	14.9	15.5	12.5
Survival index at D 1 (%)	98.8	100	100	99.2	-	-	-	-	-
Survival index at D 4 (%)	98..8	98.8	100	99.2	-	-	-	-	

- *Sex ratio:*

Table 85: Sex ratio

Exposure level (ppm)	0	25	50	100
Sex ratio M:F	46:54	51:49	48:52	51:49

- *Viability index (pups surviving 4 days/total births):*
  - *Gestation survival index :* 98.8 (168/170), 99.4 (171/172), 99.3 (151/152) and 99.2 (119/120) % resp. at 0, 25, 50 and 100 ppm
  - *Survival index at D1 :* 98.8 (166/168), 100 (171/171), 100 (151/151) and 99.2 (118/119) % resp. at 0, 25, 50 and 100 ppm
  - *Survival index at D4 :* 98.8 (166/168), 98.8 (169/171), 100 (151/151) and 99.2 (118/119) % resp. at 0, 25, 50 and 100 ppm

- *Survival index at weaning:* /
- *Mean litter or pup weight by sex and with sexes combined:*

Table 86: Pups mean body weight

Exposure level (ppm)		0	25	50	100	HCD Study # & year	1-2000	2-2003	3-2004	4-2004
Weight at D 1	♀	6.3 ± 0.4	6.5 ± 0.5	6.2 ± 0.4	6.9* ± 0.5	-	6.9	6.5	6.6	7.0
	♂	6.7 ± 0.4	6.9 ± 0.6	6.6 ± 0.6	7.3* ± 0.6	-	7.3	7.0	7.0	7.4
Weight at D 4	♀	8.8 ± 0.6	9.2 ± 0.8	8.6 ± 0.9	9.7* ± 0.9	-	9.8	9.1	9.1	10.1
	♂	9.2 ± 0.6	9.7 ± 0.8	9.2 ± 0.8	10.4* ± 0.9	-	10.2	9.6	9.7	10.7

- *External, soft tissue and skeletal malformations and other relevant alterations:* not reported
- *Number and percent of fetuses and litters with malformations (including runts) and/or variations as well as description and incidences of malformations and main variations (and/or retardations):* no reported
- *Data on physical landmarks in pups and other postnatal developmental data:* not reported
- *Data on functional observations:* no significant difference in the sensory evaluation, in the hindlimb and forelimps grip performances, or in motor activity*:* not reported

3.10.2 Human data

No human data available

3.10.3 Other data (e.g. studies on mechanism of action)

No other data available

3.11 Specific target organ toxicity – single exposure

Hazard class not evaluated in this CLH dossier

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Animal data on NITROMETHANE

3.12.1.1.1 16-day repeated dose toxicity study in rat (NTP, 1997)

Study reference:

National Toxicology Program. 1997. Toxicology and carcinogenesis studies of nitromethane (CAS No. 75-52-5) in F344/N rats and B6C3F1 mice. National Toxicology Program Technical Report Series No. 461. U.S. Department of Health and Human Services (USDHHS), Public Health Service, National Institute of Health (NIH), NIH Publication No. 97-3377.

Detailed study summary and results: Groups of 5 rats were exposed by inhalation to either 0, 94, 188, 375, 750 and 1500 ppm (equivalent to 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L resp.) nitromethane for 16 days. Clinical signs and body weight were observed weekly. At the termination of the study, all rats were necropsied for clinical pathology evaluation (heart, right kidney, liver, lungs, right testis, thymus, thyroid were weighed and plus the sciatic nerve were examined). The LOAEC was set at 375 ppm.

Test type

- sub-acute toxicity study: inhalation, 16 days
- No guideline
- Non GLP
- Not available in the registration dossier, only 90 days study available in the registration dossier but 16 days documented in the same report (NTP, 1997)

Test substance

- Nitromethane
- *Degree of purity:* >98 %
- *Impurities:* unknown

Test animals

- *Species/strain/sex:* rat / Fischer 344 / both sexes
- *Nb. of animals per sex per dose:* 5
- *Age and weight at the study initiation:* 5-week old

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration and frequency of test/exposure period:* 6-h treatment/day, for 5d/week during 16 days
- *Doses/concentration levels:* 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L equivalent to 0, 94, 188, 375, 750 and 1500 ppm, resp.
- *Post exposure observation period:* /
- *Vehicle:* air
- *Actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:*

Table 87: Actual exposure to nitromethane

Target dose (ppm)	**94**	**188**	**375**	**750**	**1500**
Actual dose (ppm)	94	187	373	748	1500
Standard Deviation (ppm)	6	10.0	19	37	58

- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* whole body
- *Analytical verification of test atmosphere concentrations:* yes
- *Particle size:* /

Results and discussion

- *Mortality and time to death:* no mortality reported
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* at 1500 ppm, in both sexes, increased preening, rapid breathing, hyperactivity at the beginning of the study and reduced activity and loss of coordination in the hindlimbs at the end of the study.
- *Body weight and body weight changes:* significant decrease in BWG in male rats exposed to 1500 ppm, in comparison with the controls. No effects on BW or BWG in females.
- *Food/water consumption:* not reported
- *Sensory activity, grip strength and motor activity assessments:* /
- *Ophthalmologic findings:* /
- *Haematological findings:* no data
- *Clinical biochemistry findings:* no data
- *Gross pathology findings:* relative liver weights in all treated male groups and absolute and relative liver weights of females exposd to 375 ppm or more were significantly increased in comparison with the controls.
- *Histopathology findings:* sciatic nerve degeneration and minimal to mild degeneration in the olfactory epithelium reported in animals of both sexes exposed to 375 ppm nitromethane and above. Reduced myelin around the sciatic nerve was also observed in the rats exposed to 750 and 1500 ppm.

3.12.1.1.2 16-day repeated dose toxicity study in mice (NTP, 1997)

Study reference:

National Toxicology Program. 1997. Toxicology and carcinogenesis studies of nitromethane (CAS No. 75-52-5) in F344/N rats and B6C3F1 mice. National Toxicology Program Technical Report Series No. 461. U.S. Department of Health and Human Services (USDHHS), Public Health Service, National Institute of Health (NIH), NIH Publication No. 97-3377.

Detailed study summary and results: Groups of 5 mice were exposed by inhalation to either 0, 94, 188, 375, 750 and 1500 ppm (equivalent to 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L resp.) nitromethane for 16 days. Clinical signs and body weight were observed weekly. At the termination of the study, all rats were necropsied for clinical pathology evaluation (heart, right kidney, liver, lungs, right testis, thymus were weighed and were examined). The LOAEC was set at 375 ppm.

Test type

- Sub-acute toxicity study: inhalation, 16 days
- No guideline
- Non GLP
- Not available in the registration dossier, only 90 days study available in the registration dossier but 16 days documented in the same report (NTP, 1997)

Test substance

- Nitromethane
- *Degree of purity:* >98 %
- *Impurities:* unknown

Test animals

- *Species/strain/sex:* mice / B6C3F1 / both sexes
- *Nb. of animals per sex per dose:* 5
- *Age and weight at the study initiation:* 5-week old

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration and frequency of test/exposure period:* 6-h treatment/day, for 5d/week during 16 days
- *Doses/concentration levels:* 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L equivalent to 0, 94, 188, 375, 750 and 1500 ppm, respectively.
- *Post exposure observation period:* /
- *Vehicle:* air
- *Actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose:*

Table 88: Actual exposure to nitromethane

Target dose (ppm)	**94**	**188**	**375**	**750**	**1500**
Actual dose (ppm)	94	187	373	748	1500
Standard Deviations (ppm)	6	10.0	19	37	58

- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* whole body
- *Analytical verification of test atmosphere concentrations:* yes
- *Particle size:* no data

Results and discussion

- *Mortality and time to death:* no mortality reported
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* reduced activity and tachypnea in both sexes at 1500 ppm
- *Body weight and body weight changes:* no effect on BW or BWG
- *Food/water consumption:* /
- *Sensory activity, grip strength and motor activity assessments:* /
- *Ophthalmologic findings:* /
- *Haematological findings:* no data
- *Clinical biochemistry findings:* no data
- *Gross pathology findings:* significant increase in absolute and relative liver weights in males mice exposed to 750 ppm or greater and in all exposed female mice. At 375 ppm, significant increase in the relative liver weight in males.
- *Histopathology findings:* degeneration of the olfactory epithelium in both sexes starting from 375 ppm (minimal in males and minimal to mild in females)

3.12.1.1.3 13-week repeated dose toxicity study in rat (NTP, 1997)

Study reference:

National Toxicology Program. 1997. Toxicology and carcinogenesis studies of nitromethane (CAS No. 75-52-5) in F344/N rats and B6C3F1 mice. National Toxicology Program Technical Report Series No. 461. U.S. Department of Health and Human Services (USDHHS), Public Health Service, National Institute of Health (NIH), NIH Publication No. 97-3377.

Detailed study summary and results: a 13-week inhalation study was performed and reproductive organs were analysed. Fischer 344 rats (males and females, 10/sex/dose) were exposed to vapour of nitromethane (purity > 98 %) at doses of 0, 94, 188, 375, 750 or 1500 ppm for 13 weeks. Clinical signs and body weight were observed weekly. Neurobehavioral testing was performed during week 11. Additional groups of 10 rats per sex were used for clinical pathology assessment (on day 3 and 23). At the termination of the study, all rats from the "core study" were also necropsied for clinical pathology evaluation. As reproductive effects, it was noted a significant decrease in sperm motility when males were exposed to 750 or 1500 ppm, in comparison with the control group. Furthermore, in the 1500 ppm group, a significant decrease in testis, epididymis and cauda weights was reported. No effects were observed on females reproductive system or estrous cycle. Reproductive organs tissues were not affected in either males or females. In males exposed to 1500 ppm, systemic toxicity was reported that might have caused secondary effects. The LOAEC (systemic, male/female) was determined as 375 ppm, the NOAEC (systemic, male/female) was 94 ppm based on disturbance of hematological parameters at 188 ppm, the LOAEC (local, male/female) was 375 ppm for the upper respiratory tract, and the NOAEC (local, male/female) was 188 ppm.

Test type

- Similar to guideline study OECD TG 413 (90-day), sub-chronic toxicity: inhalation
- GLP-compliant
- Reliability 1 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* >98 %
- *Impurities:* unknown

Test animals

- *Species/strain/sex:* rat / Fischer 344 / both sexes
- *Nb. of animals per sex per dose:* 10
- *Age and weight at the study initiation:* 4-week old

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration and frequency of test/exposure period:* 6-h12min treatment/day, for 5d/week during 13 weeks
- *Doses/concentration levels:* 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L equivalent to 0, 94, 188, 375, 750 and 1500 ppm, respectively.
- *Post exposure observation period:* /
- *Vehicle:* air
- *Actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose:*

Table 89: Actual exposure to nitromethane

Target dose (ppm)	**94**	**188**	**375**	**750**	**1500**
Actual dose (ppm)	94	187	373	748	1500
Standard Deviation (ppm)	6	10.0	19	37	58

- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* whole body
- *Analytical verification of test atmosphere concentrations:* yes
- *Particle size:* no data

Results and discussion

- *Mortality and time to death:* no mortality reported
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* hindlimbs paralysis in all animals (both sexes) exposed to 1500 ppm starting from D21 and in 1/10 male and 4/10 females exposed to 750 ppm starting on D 63.

- *Body weight and body weight changes:* Significant decrease in FBW and BWG, in comparison with the control group, in males exposed to 1500 ppm

Table 90: BW and BWG

Exposure level (ppm)		0	94	188	375	750	1500
♂	BW at start	107 ± 3	105 ± 2	113 ± 2	109 ± 3	106 ± 2	109 ± 2
	Final BW	334 ± 7	323 ± 7	345 ± 4	336 ± 5	327 ± 4	295 ± 10**
	BWG	228 ± 6	218 ± 7	232 ± 3	227 ± 4	221 ± 5	185 ± 9**
♀	BW at start	95 ± 1	96 ± 2	97 ± 2	95 ± 2	96 ± 2	94 ± 2
	Final BW	185 ± 5	197 ± 3	197 ± 3	198 ± 5	194 ± 4	177 ± 4
	BWG	90 ± 3	101 ± 2	100 ± 2	103 ± 4**	97 ± 2	84 ± 3

- *Food/water consumption:* /
- *Sensory activity, grip strength and motor activity assessments:* hindlimbs paralysis in 100 % rats exposed to 1500 ppm, in both sexes, starting from day 21. Significant decrease in hindlimbs and forelimbs strength in males exposed to 1500 ppm and in females (only hindlimbs strength) in the 750 and 1500 ppm groups, in comparison with the controls
- *Ophthalmologic findings:* /
- *Haematological findings:* dose-dependent microcytic responsive anemia (with decreased Hb concentration at all time points in all animals exposed to 375, 750 and 1500 ppm and at several time points at 94 and 188 ppm); increase in methemoglobin concentration at 1500 ppm in both sexes
- *Clinical biochemistry findings:* decrease in T3, thyroxine and free thyroxine in animals exposed to 1500 ppm, in both sexes, seen at day 23
- *Gross pathology findings:* some organ weights were decreased at 1500 ppm. No significant changes in organ weights
- *Reproductive data:* no significant change in the length of the estrous cycle, but significant decrease in the sperm motility at 750 and 1500 ppm

Table 91: Reproductive data

Exposure level (ppm)		0	375	750	1500
Males					
Nb		10	10	10	10
Sperm parameters	Motility	94.57 ± 1.30	92.16 ± 1.90	87.11 ± 1.88**	76.43 ± 2.78**
	Count (mean/10^{-4} mL suspension)	64.33 ± 3.89	62.75 ± 3.63	62.68 ± 3.02	68.95 ±3.14
Weights (g)	Final BW at termination	338 ± 7	341 ± 4	331 ± 4	299 ± 11**

	L. cauda	0.207 ± 0.004	0.210 ± 0.004	0.204 ± 0.006	0.177 ± 0.009**
	L. epididymis	0.467 ± 0.009	0.468 ± 0.006	0.444 ± 0.009	0.412 ± 0.013**
	L. testis	1.39 ± 0.03	1.36 ± 0.01	1.34 ± 0.02	1.29 ± 0.02**
Females					
Nb		10	10	10	10
Weight (g)	At termination	188 ± 5	200 ± 5	195 ± 4	178 ± 3
Estrous cycle length	In days	4.89 ± 0.07a	4.75 ± 0.16b	5.00 ± 0.14a	5.00 ± 0.15

**p<0.01; a: estrous cycle greater than 12d in 1/10 female, b: estrous cycle greater than 12d in 2/10 females

- *Histopathology findings:* non-neoplastic findings observed in several tissues of animals exposed to 1500 ppm

Table 92: Non-neoplastic lesions

Exposure level (ppm)		**0**	**94**	**188**	**375**	**750**	**1500**
♂	**Nb**	10	10	10	10	10	10
	Bone marrow hyperplasia	0	0	0	0	9**	10**
	Degeneration olf. epithelium	0	No animal tested	0	9**	10**	10**
	Hyaline droplets, olf. epithelium	0	No animal tested	0	0	1	8**
	Hyperplasia Goblet cells	0	No animal tested	0	0	1	10**
	Sciatic nerve degeneration	0	No animal tested	0	5*	10**	10**
	Spinal cord degeneration	0	No animal tested	0	9**	10**	10**
♀	**Nb**	10	10	10	10	10	10
	Bone marrow hyperplasia	0	0	1	6**	7**	10**
	Degeneration olf. epithelium	0	0	1	10**	10**	10**
	Hyaline droplets, olf. epithelium	0	0	0	0	4*	10**
	Hyperplasia Goblet cells	0	0	0	0	2	10**
	Sciatic nerve degeneration	0	No animal tested	0	8**	10**	10**
	Spinal cord degeneration	0	No animal tested	0	2	10**	10**

- *NOAEC(male):* 375 ppm; LOAEC(male): 750 ppm (decreased sperm mobility)
- *NOAEC(female*): >1500 ppm

3.12.1.1.4 13-week repeated dose toxicity study in mouse (NTP, 1997)

Study reference:

National Toxicology Program. 1997. Toxicology and carcinogenesis studies of nitromethane (CAS No. 75-52-5) in F344/N rats and B6C3F1 mice. National Toxicology Program Technical Report Series No. 461. U.S. Department of Health and Human Services (USDHHS), Public Health Service, National Institute of Health (NIH), NIH Publication No. 97-3377.

Detailed study summary and results: a 13-week inhalation study was performed and reproductive organs were analysed. B6C3F1 mice (males and females, 10/sex/dose) were exposed to vapour of nitromethane (purity > 98 %) at doses of either 0, 94, 188, 375, 750 or 1500 ppm. Clinical signs and body weight were observed weekly. Additional groups of 5 mice per sex were used for parasite and clinical pathology assessment (before the study started) and the kidneys of 5 mice/sex were removed and evaluated. At the termination of the study, a serologic examination was performed on 5 mice/sex and all mice were also necropsied for clinical pathology evaluation. No effects were seen on final body weights in either sex, on cauda, epididymis or testis weights, or on sperm count. However, in males, the sperm motility was significantly decreased at 375, 750 and 1500 ppm, in comparison with the control group. In females, the estrous cycle length was dose-dependently increased starting from 375 ppm, in comparison with the controls. The LOAEC (systemic, male/female) was determined as 188 ppm based on the modification of some organ weights, the NOAEC (systemic, male/female) was 94 ppm based on the effects seen at 188 ppmon organ weights, the LOAEC (local, male/female) was 375 ppm for the upper respiratory tract, and the NOAEC (local, male/female) was 188 ppm.

Test type

- Equivalent to OECD TG 413
- GLP-compliant
- Reliability 1 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* >98 %
- *Impurities:* unknwon

Test animals

- *Species/strain/sex:* mouse / B6C3F1 / both sexes
- *Nb. of animals per sex per dose:* 10
- *Age and weight at the study initiation:* 4-wek old

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration and frequency of test/exposure period:* 6-h treatment/, for 5d/week during 13 weeks

- *Doses/concentration levels:* 0, 94, 188, 375, 750 and 1500 ppm equivalent to 0, 0.235, 0.47, 0.938, 1.88 or 3.75 mg/L
- *Post exposure observation period:* /
- *Vehicle:* air
- *Actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose:*

Table 93: Actual exposure to nitromethane

Target dose (ppm)	**94**	**188**	**375**	**750**	**1500**
Actual dose (ppm)	93.6	187	373	748	1500
Standard Deviation (ppm)	5.5	10.0	19	37	58

- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* whole body
- *Analytical verification of test atmosphere concentrations:* yes
- *Particle size:* /

Results and discussion

- *Mortality and time to death:* no mortalty occured
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* no data
- *Body weight and body weight changes:* no effects in males, statistically significant increase in terminal BW in females at 375 ppm, but no changes at the other doses.
- *Food/water consumption:* not specified
- *Sensory activity, grip strength and motor activity assessments:* /
- *Ophthalmologic findings:* /
- *Haematological findings:* no effects
- *Clinical biochemistry findings:* no effects
- *Reproductive data:* Dose-related decrease in sperm motility was reported in males exposed to 375, 750 and 1500 ppm. In females, estrous cycle length increased in a dose-dependent way starting from 375, 750 and 1500 ppm.

Table 94: Sperm motility

Exposure level (ppm)	0	375	750	1500
Motility (%)	93.50 ± 0.46	85.09 ± 1.21**	86.47 ± 1.17**	82.42 ± 1.30**

Table 95: Estrous cycle length

Exposure level (ppm)	0	375	750	1500
Length in days	4.00 ± 0.00 [a]	4.33 ± 0.14* [b]	4.50 ± 0.21*	4.71 ± 0.26**[c]

a = cycle > 12d or unclear in 2/10 mice, b = cycle > 12d or unclear in 1/10 mice, c = cycle > 12d or unclear in 3/10 mice

- *Gross pathology findings:* In males, increase of the relative liver and right kidney weights at 1500 ppm, in comparison with the controls. In females, increase of the relative and absolute weights of kidneys at 1500 ppm, in comparison with the controls. No effects on heart, lung, testis and thymus relative or absolute weights in males; no effects on liver, lung, thymus relative or absolute weights in females. In females, heart relative weight was significantly decreased at 375 ppm, in comparison with the controls, but not at lower or higher dose.

Table 96: Organ weights

Dose level (ppm)		0	94	188	375	750	1500
Males							
Liver	Abs	1.633 ± 0.040	1.700 ± 0.023	1.678 ± 0.031	1.731 ± 0.027	1.789 ± 0.029*	1.724 ± 0.053
	Rel	45.27 ± 0.89	47.32 ± 0.38	47.39 ± 0.78	47.70 ± 0.60*	50.79 ± 0.72**	49.62 ± 0.99*
Kidney	Abs	0.294 ± 0.009	0.329 ± 0.006**	0.322 ± 0.005*	0.332 ± 0.007**	0.339 ± 0.007**	0.315 ± 0.008
	Rel	8.15 ± 0..20	9.15 ± 0.11**	9.10 ± 0.15**	9.15 ± 0.20**	9.63 ± 0.20**	9.08 ± 0.18**
Females							
Kidney	Abs	0.210 ± 0.007	0.221 ± 0.005	0.228 ± 0.005*	0.232 ± 0.005*	0.231 ± 0.006*	0.230 ± 0.006*
	Rel	6.75 ± 0.18	7.03 ± 0.15	6.97 ± 0.15	6.80 ± 0.17	7.33 ± 0.21*	7.57 ± 0.15**

- *Histopathology findings:* at 1500 ppm, hyaline droplets and degeneration were spotted in the respiratory epithelium as well as extramedullary hematopoiesis in the spleen.

Table 97: Non-neoplastic lesions

Exposure level (ppm)		**0**	**94**	**188**	**375**	**750**	**1500**
♂	**Nb**	10	10	10	10	10	10
	Degeneration olf. epithelium	0	0	0	10**	10**	10**
	Hyaline droplets, olf. epithelium	0	0	1	10**	10**	10**
	Extramedull. Hematopoiesis (spleen)	0	1	0	1	2	10**
♀	**Nb**	10	10	10	10	10	10

	Degeneration olf. epithelium	0	0	7**	10**	10**	10**
	Hyaline droplets, olf. epithelium	0	2	9**	10**	10**	10**
	Extramedull. Hematopoiesis (spleen)	0	0	0	2	3	9**

- *LOAEC(male/female):* 375 ppm

3.12.1.1.5 Subchronic inhalation toxicity study in rat (Lewis *et al.*, 1977)

Study reference:

Lewis T.R. *et al.*, 1977. Subchronic inhalation toxicity of nitromethane and 2-nitropropane, J Eanvironmen Pathol Toxicol 2, 233-249.

Detailed study summary and results: Male rats were exposed by inhalation to 100 and 750 ppm nitromethane for 13 weeks, and up to 24 weeks. Body weights and body weight gains were followed up regularly. 10 Animals from each dose group were sacrificed by phenobarbital overdose and exsanguinated at different time points where blood hematology and biochemistry as well as several tissue examinations (lungs, liver, kidney, trachea, brain, thyroid) were analysed (after 2 d, 10 d, 1 month, 3 months, 6 months). The LOAEC (male) was 745 ppm based on a decrease in BWG after 2 months of exposure and the NOEC was 98 ppm.

Test type

- Not following guideline
- Not GLP-compliant
- Reliability 2 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* 96.5 %
- *Impurities:* 1.5 % nitroethane, 1.4 % nitropropane, 0.5 % propionitrite

Test animals

- *Species/strain/sex:* rat / SD / male
- *Nb. of animals per sex per dose:* 50 male rats
- *Age and weight at the study initiation:* 100 g in average, age unknown

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration and frequency of test/exposure period:* 7h/d, 5d/wk, for 13 weeks and up to 24 weeks
- *Doses/concentration levels:* 0, 100 and 750 ppm (equivalent to 0.25 and 1.875 mg/L, resp.)
- *Post exposure observation period:* /
- *Vehicle:* air

- *Actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose:* the target doses were 100 and 750 ppm while the actual dose of exposure were 97.6 ± 4.6 ppm and 745.0 ± 34.0 ppm, resp.
- *Statistical methods:* Bartlett's test for homogeneity of variance (rejection level at p = 0.01), then one-way analysis of variance (rejection level p=0.1), then Student's t test when significance was indicated (rejection level at p=0.05)
- *Type of inhalation exposure and test conditions (e.g.: exposure apparatus):* exposure chambers
- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* whole body
- *Analytical verification of test atmosphere concentrations:* yes
- *Particle size:* /

Results and discussion

- *Mortality and time to death (if occurring)*
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* not specified
- *Body weight and body weight changes:* BWG decreased in rats exposed to 750 ppm: starting from the 8th week, a decrease in BWG was observed, in comparison with the control group. The decrease was significant except during week 13. No effects on BW in rats exposed to 100 ppm, compared to controls.
- *Food/water consumption:* no data
- *Sensory activity, grip strength and motor activity assessments:* /
- *Ophthalmologic findings:* /
- *Haematological findings:* hematocrit level was significantly decreased in rats exposed to 750 ppm at all time points, except at day 2. When exposed to 100 ppm, the hematocrit level was only decreased at the day 10 time point. Hemoglobin level was significantly decreased at all time points when rats were exposed to 750 ppm, however, in rats exposed to 100 ppm, the decrease was only seen at the day 10 time point. Red blood cells counts increased in the group exposed to 750 ppm at the 2-day time point, but they were decreased at the day 10, 1-month and 3-month time points. The difference with the control group was not significant only at the day 10 time point. When rats were exposed to 100 ppm, the red blood cells counts were only increased at the 10-day time point, compared to controls. Methemoglobin and prothrombin concentrations were not modified in both treatment groups.

Table 98: Hematological parameters

Parameters	Dose level in ppm	Day 2	Day 10	Month 1	Month 3	Month 6
Ht	0	39 ± 0.5	41 ± 0.5	44 ± 0.3	44 ± 0.7	43 ± 0.5
	750	40 ± 0.9	39 ± 0.9*	42 ± 0.4***	41 ± 0.3***	40 ± 0.8**

Hb	0	10.8 ± 0.22	13.9 ± 0.21	14.6 ± 0.13	14.8 ± 0.23	14.0 ± 0.23
	750	11.1 ± 0.21	12.9 ± 0.25***	13.7 ± 0.17***	13.0 ± 0.22***	12.3 ± 0.22***
RBC	0	5.61 ± 0.111	6.31 ± 0.97	6.89 ± 0.112	6.47 ± 0.123	7.79 ± 0.127
	750	6.03 ± 0.123*	5.89 ± 0.116*	6.68 ± 0.064	6.05 ± 0.068**	7.71 ± 0.128
MetHb	0	0 ± 0.1	0.08 ± 0.007	0.06 ± 0.008	0.08 ± 0.022	0.01 ± 0.002
	750	0 ± 0.1	0.08 ± 0.006	0.10 ± 0.029	0.08 ± 0.011	0.07 ± 0.058
PT time	0	15.1 ± 1.17	14.2 ± 0.12	15.1 ± 0.49	15.8 ± 0.31	14.6 ± 0.28
	750	16.8 ± 1.58	13.7 ± 0.20*	14.6 ± 0.25	15.6 ± 0.26	14.8 ± 0.34

With * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$

- *Clinical biochemistry findings:* OCT levels were increased at the 10-day time point in rats exposed to 750 ppm. T4 concentrations were reduced at the 2-day time point in rats
- *Gross pathology findings:* After a 2-day, 10-day and 1-month exposure to nitromethane, no macroscopic effects were seen at both doses. At the 3-month time point, “whitish or greyish” focal areas in the lung were seen in both exposure groups. At the 6-month time point, a significant increase in the incidence of white focal areas scattered on all lungs lobes of the exposed and control group was reported as well as a decrease in the number of focal hemorrhages on the lungs. Pale kidneys were also reported in control and treated groups. Concerning organ weights, the lung weights tended to decrease at all time points. At the 6-month time point, the thyroid gland weights were increased in the group exposed to 750 ppm, in comparison with the controls.
- *Histopathology findings:* No lung or brain edema were reported in treated rats, for both doses. Microscopic alterations were dispersed in several tissues in control and treated groups. Extramedullary hematopoiesis was reported in the spleen of control and treated groups. Some dispersed focal nonsuppurative areas of pneumonitis were reported in lungs of rats from the control and treated groups. At the 6-month time point, dispersed microscopic alterations were observed in the spleen and the kidneys: in the spleen, extramedullary hematopoieses and pigmented areas were seen in control and treated groups, while in the kidneys, mild nephritis was evidenced in some animals.

3.12.1.1.6 Subchronic inhalation toxicity study in rabbit (Lewis *et al.*, 1977)

Study reference:

Lewis T.R. *et al.*, 1977. Subchronic inhalation toxicity of nitromethane and 2-nitropropane, J Environmen Pathol Toxicol, 2, 233-249.

Detailed study summary and results: groups of 5 rabbits were exposed to 0, 100 or 750 ppm (target doses: 100 or 750 ppm, resp.) nitromethane by inhalation. A clinical examination as well as blood testing and

histopathological assessment were performed at various time points (1, 3 and 6 months). The LOAEC (male) was 98 ppm based on a reduction in T4 levels throughout the study.

Test type

- Not following guidelines
- GLP-compliance not specified
- Reliability 2 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* 96.5 %
- *Impurities:* 1.5 % nitroethane, 1.4 % 2-nitropropane, 0.5 % propionitrile

Test animals

- *Species/strain/sex:* rabbit / NZW / male
- *Nb. of animals per sex per dose:* 15 males
- *Age and weight at the study initiation:* not specified

Administration/exposure

- *Route of administration:* inhalation (vapour)
- *Duration and frequency of test/exposure period:* 7h/d, 5d/wk, for 6 months
- *Doses/concentration levels:* 100 and 750 ppm equivalent to 0.25 and 1.875 mg/L, resp.
- *Post exposure observation period:* /
- *Vehicle:* air
- *Actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose:* 97.6 ± 4.6 and 745 ± 34 ppm instead of 100 and 750 ppm, resp.
- *Statistical methods:* non parametric tests: Kruskal-Wallis one-way analysis of variance (rejection level p=0.10), if differences were indicated then a Mann-Whitney U test was performed (p=0.05)
- *Type of inhalation exposure and test conditions (e.g.: exposure apparatus):* chambers
- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* whole body
- *Analytical verification of test atmosphere concentrations:* yes
- *Particle size:* /

Results and discussion

- *Mortality and time to death:* no mortality occured
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* no info
- *Body weight and body weight changes:* no effects
- *Food/water consumption:* not specified
- *Sensory activity, grip strength and motor activity assessments:* /

- *Ophthalmologic findings:* /
- *Haematological findings:* hemoglobin levels were reduced at 1 month. No effects were seen on the erythrocytes count, hematocrit, methemoglobin and prothrombin levels.
- *Clinical biochemistry findings:* T4 levels were reduced throughout the study, at both doses. The decrease was statistically significant at 1-month time points in animals exposed to 750 ppm and at the 6 months time point in both exposed groups. OCT levels increased at 1 and 3 months, at both dose levels, however the serum levels were inferior to control values at 6 months.
- *Gross pathology findings:* thyroid gland weights were increased after 6 months of exposure. As no more information is available, it is supposed that this effect appeared at both doses
- *Histopathology findings:* At the 1-month time point, modifications were seen in the lungs as focal aeras of mild to severe haemorrhage and congestion of the alveolar area and duct walls. Edema and sometimes necrosis were seen in the congestioned or bleeding areas. Lung edema was also reported in some animals. Nonsuppurative pericholangitis and nonsuppurative focal encephalitis were observed in control and exposed groups.

3.12.1.1.7 Subchronic oral repeated dose toxicity study (Weatherby *et al.*, 1955)

Study reference:

Weatherby J.H. *et al.*, 1955. Observations on the Toxicity of Nitromethane, AMA Archives of Industrial Health, 11, 102-106.

Detailed study summary and results: male rats were exposed to nitromethane in drinking water for 15 weeks. Doses chosen were 0, 0.1, 0.25, 0.5, 1 and 2 %. Only the control, 0.1 and 0.2 % groups were kept after a week of test because animals did not take concentrations superior or equal to 0.5 %. Several animals died (3 and 4 at 0.25 and 0.1 % groups, resp.). In surviving animals, necropsy was performed and tissues were examined. A the end of exposure period, daily fluid intake (and then nitromethane exposure) was calculated, gross and microscopic changes were assessed in the heart, lungs, liver, spleen, kidney, testes, adrenal gland and small intestine.

Test type

- Not following guidelines
- Not GLP-compliant
- Disregarded due to methodological deficiencies
- Reliability 3 (according to the registration dossier)

Test substance

- Nitromethane
- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* rat / albino / male

- *Nb. of animals per sex per dose:* 10 male rats/dose
- *Age and weight at the study initiation:* young, between 40 and 60 g

Administration/exposure

- *Route of administration:* oral
- *Duration and frequency of test/exposure period:* continuous for 15 weeks
- *Doses/concentration levels:* 0, 0.1, 0.25, 0.5, 1 and 2 % nitromethane in drinking water
- *Post exposure observation period:* /
- *Vehicle:* water
- *Control group and treatment:* yes, only water
- *Actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable:*based on a graph, the dossier submitter interpreted that the actual intake was as follow:

Table 99: Actual exposure to nitromethane (NM) through drinking water (in mg/kg bw/d)

	Max. dose ingested	**Daily ingestion (at the end of the study period)**	**Average daily intake**
0.1 % NM	200	70	150
0.25 % NM	385	170	285

Results and discussion

- *Mortality and time to death:* 4 and 3 animals out of 10 died in groups exposed to 0.1 and 0.25 %, respectively
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* not specified
- *Body weight and body weight changes:* decreased BW at 0.1 and 0.25 % (no more info)
- *Food/water consumption:* decrease in water consumption (and nitromethane absorption) through the study
- *Sensory activity, grip strength and motor activity assessments (when available):* /
- *Ophthalmologic findings:* /
- *Haematological findings:* /
- *Clinical biochemistry findings:* /
- *Gross pathology findings:* /
- *Histopathology findings:* liver cells cytoplasm less stained in 6/7 animals in the group exposed to 0.25 % nitromethane, in comparison with the control group, and more lymphocytes were noted in the periportal zone. Hepatic cells were larger and their nucleus prominent in 2/6 surviving animals in the group exposed to 0.1 % nitromethane. 1/10 rats in the control group had large hepatic cells with

prominent nuclei. In the group exposed to 0.25 % nitromethane, 2/7 surviving animals had more prominent Malpighian corpuscles compared to normal spleen.

In animals exposed to 0.5 % and above for a week, only animals exposed to 2 % nitromethane developed lesions in the liver (numerous lymphocytes in periportal area and staining was not as deep as in controls).

3.12.1.2 Animal data on NITROETHANE

3.12.1.2.1 13-week repeated dse inhalation toxicity study in rat (Anonymous 26, 1982)

Study reference:

Anonymous 26, 1982

Detailed study summary and results: The subchronic toxicity of nitroethane was examined in rats. Groups of rats were exposed to 0, 100, 350 or 1000 ppm (equivalent to 0, 0.3, 1.0 or 3.0 mg/L) of nitroethane for 6 h/d, 5 d/week for a total of a 92-d period with an interim sacrifice of rats after a 30-day period. Parameters monitored were clinical observations, body weights, organ weigths, hematological characteristics including methemoglobin (MetHb) determination, clinical chemistries, urinalysis, gross pathology and histopathology. The LOAEC was set at 100 ppm for males and females based on histopathologic changes in the salivary gland after 13 weeks exposure.

Test type

- Equivalent or similar to OECD TG 413 (Major deviation : feed consumption was not measured)
- Study was initiated prior to GLP and completed with GLP
- Reliability 2 (according to the registration dossier)

Test substance

- Nitroethane
- *Degree of purity:* >97 %
- *Impurities:* Nitromethane < 1 %; 2-Nitropropane < 1.5 %

Test animals

- *Species/strain/sex:* Rat / Fischer 344 / both sexes
- *Nb. of animals per sex per dose:* 10/sex/dose + interim group of 5/sex/dose
- *Age and weight at the study initiation:* 9 weeks old, BW not specified

Administration/exposure

- *Route of administration:* inhalation (vapours)
- *Duration and frequency of test/exposure period:* 6 h/day, 5 d/week (excluding holidays) for 30 d (5/sex/dose, interim group) or for 92 d (10/sex/dose)
- *Doses/concentration levels:* 0, 100, 350 or 1000 ppm (corresponding to 0, 0.3, 1.0 or 3.0 mg/L)
- *Post exposure observation period:* /

- *Vehicle:* air
- *Control group and treatment:* Sham-exposed animals
- *Statistical methods:* Analysis of variance and Dunnett's test using a level of significance of $p < 0.05$
- *Type of inhalation exposure and test conditions (e.g.: exposure apparatus):* 1 cubic meter stainless steel and glass Rochester-type chamber under dynamic airflow conditions. (Airflow 175 i/min, Temperature 70°F, relative humidity 50 %)
- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* whole body
- *Analytical verification of test atmosphere concentrations:* Infrared spectrophotometer equipped with a variable pathlength gas cell. Wavelength 11.5 microns. Analysis performed 1-2 times per hour for each exposure concentration.

Results and discussion

Rats were exposed during 13 weeks to 0, 100, 350 or 1000 ppm nitroethane. When exposed to the high dose level, a decreased in rats BW gain was observed, as well as an increase in methemoglobin levels (associated with cyaniosis), in reticulocytes and Heinz bodies in blood associated with splenic congestion and extramedullary hematopoiesis. Degenerative and inflammatory modifications were seen in nasal epithelium, vacuolization of hepatocytes, reduced cytoplasmic granularity of kidney cortical tubular epithelial tissue and ductal epithelial cells in the salivary glands. At the middle dose, same changes, although to a lesser intensity, were observed in ethemoglobin levels, spleen, nasal epithelium and salivary glands. The changes were minimal at 100 ppm in the methemoglobin level, spleen and salivary glands.

- *Mortality and time to death:* no mortalty occured
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* Two clinical findings, cyanosis and red eyes, were consistent with the grossly observable treatment-induced methemaglobinemia
    - Dull, dark red eyes: very pronounced in the 1000 ppm group (appeared after the first exposure and thereafter) , not very distinctive in the 350 ppm group (appeared after 4 weeks of exposure)
    - Grayish or bluish colored skin of the extremities (cyanosis): in the 350 ppm group after 9 weeks of exposure and in the 1000 ppm group after 4 exposure and thereafter. Disappear within 19 hours after exposure each time
    - Female rats of the 100, 350 and 1000 ppm exposure groups had an unkept appearance which was an expression of their general weakened condition secondary to the toxicity of the test material.

  Two other clinical findings, swelling in the salivary gland region and increased amounts of porphyrin pigments around the nares, were observed in some rats of the 100, 350 or 1000 ppm group. These observations were consistent with a mild transient viral infection

(sialodacryoadenitis) which commonly occurs in this laboratory and were not judged to be treatment-related.

- *Body weight and body weight changes:* Growth retardation in the 1000 ppm and 350 ppm female and male rat. All of these treatment groups had statistically significant body weight decreases when compared to controls during the last month of the study, despite the fact that the 1000 ppm female rats weighted statistically significantly less than their controls prior to the start of the study. Group mean body weight for both sexes of the 100 ppm group were comparable to their controls.

Table 100: Rat body weights data (in g)

0	100	350	1000	Exposure level (ppm)		0	100	350	1000
Males				Exposure day	Experiment day	**Females**			
158±4	159±6	175±6	159±7	-1	-1	110±5	106±4	109±4	102±9*
178±10	175±8	168±8	156±6	2	2	121±5	117±4	116±4	100±6
185±8	179±10	178±8	162±7	4	6	126±5	121±4	119±5	107±7
197±8	188±11	190±9	177±8	7	9	133±6	130±4	130±5	118±7
207±9	198±11	197±9	188±9	9	13	141±6	135±5	133±4	125±7
233±11	223±12	224±10	212±10	14	20	153±6	147±5	143±4	136±5
248±11	244±8	240±10	231±9	19	27	163±7	156±5	151±5	142±6
257±10	256±7	248±10	237±10	24	33	167±7	161±7	153±6	146±7
275±10	272±7	265±7	250±12	29	40	173±7	170±8	162±8	152±6
286±11	285±9	275±10	259±15	34	47	180±8	173±9	164±6	154±8
298±13	297±8	287±11	271±11	39	54	187±9	178±9	171±9	161±7
309±12	307±9	298±13	277±7*	44	61	191±8	186±10	177±7*	166±6*
322±13	315±7	304±13*	282±7*	49	68	194±10	186±9	176±9*	168±6*
328±16	321±9	313±12*	286±8*	54	75	198±9	189±7*	178±8*	169±5*
330±15	315±18	321±13	292±8*	57	82	191±7	185±9	182±7*	172±6*
326±14	322±20	316±11	293±8*	62	90	194±10	190±10	184±7*	176±7*

- *Food/water consumption:* not measured
- *Sensory activity, grip strength and motor activity assessments:* /
- *Ophthalmologic findings:* /
- *Haematological findings:*
  - *Prior to the interim kill (30 days):* in the 1000 ppm group, statistically significantly lowered hemoglobin values in male rats and statistically significant increases of the WBC counts ; in the 350 and 1000 ppm groups, increased emergence of reticulocytes and Heinz bodies
  - *Prior to the terminal kills (92 days):* in the 1000 ppm group, statistically significantly increased PCV and a decreased RBC count in females and statistically significantly lowered

hemoglobin values in male rats; in the 350 and 1000 ppm groups, increased emergence of reticulocytes and Heinz bodies

Table 101: Hematological parameters

Males				**Exposure (ppm)**	Females			
0	100	350	1000		0	100	350	1000
At interim kill								
51.2±2.2	49.1±0.9	49.9±2.4	48.8±2.2	PCV	46.7±2.0	47.9±1.7	48.0±1.2	49.4±2.6
8.47±0.44	8.14±0.27	8.49±0.57	7.79±0.58	RBC	7.83±0.37	7.73±0.33	8.11±0.28	7.41±0.13
16.7±0.4	16.4±0.6	16.2±0.3	15.0*±0.4	Hb	15.9±0.7	15.9±0.5	16.1±0.6	16.0±0.4
12.4±1.6	11.3±0.9	11.6±1.1	15.0*±1.8	WBC	12.5±1.1	12.2±1.8	13.5±1.3	19.6*±2.3
1.7±0.8	1.4±0.9	2.8±1.3	2.8±1.4	Ret.	1.5±0.7	1.5±0.6	1.6±0.5	2.0±0.5
0.3±0.1	0.4±0.2	1.2*±0.2	1.9*±0.8	Heinz bodies	0.5±0.2	0.4±0.2	0.8±0.2	2.6*±0.4
At terminal kill								
52.9±1.5	48.8*±2.3	48.4*±2.2	52.1±2.2	PCV	50.6±1.3	48.6±1.7	47.9*±2.2	56.4*±1.6
9.00±0.36	8.43±0.34	8.42±0.45	7.99*±0.60	RBC	8.38±0.31	7.85*±0.22	7.93*±0.29	8.15±0.23
17.0±0.5	16.2±0.5	16.2±0.5	16.4±0.7	Hb	16.8±0.3	16.0*±0.5	16.0*±0.6	18.1*±0.2
10.7±1.0	12.0±1.6	13.8*±2.0	15.0*±2.4	WBC	10.3±3.0	12.4±1.8	10.3±2.2	13.7*±2.4
0.2±0.2	0.5±0.5	0.9±0.4	2.7*±1.0	Ret.	0.4±0.4	1.3±0.8	1.1±0.7	4.0*±2.5
0.4±0.4	0.5±0.3	1.5±0.8	10.0*±2.2	Heinz bodies	0.2±0.2	0.3±0.2	1.0±0.5	6.4*±1.9

PCV= packed cells volume (%); RBC= Red blood cells (x10^6/mm^3); Hb= Hemoglobin (g/100mL); WBC= White blood cells (x10^3/mm^3); Reticulocytes (%); Heinz bodies (%)

- *Methemoglobinemia:* prior to interim kill (20th exposure day, D 29 of the experiment), methemoglobin was dosed in blood, 15 hours after the last exposure (Part A of next Table). All exposed rats had a methemoglobinemia level comparable to control animals. Nonetheless, complementary analysis of hemoglobinemia was performed when dull dark red eyes and bluish skin in rats exposed to 1000 ppm were objectified. These clinical signs were transient and were disappeared by the next morning. According to the registrant, females seemed to be more affected than males and an experiment just after exposure was performed only for the control group and females exposed to the

highest dose. The increase seen in females methemoglobinbemia was severely significant compared to controls, and the registrant concluded that the time of analysis was a key element to characterize nitroethane effects on methemoglobinemia (Part B of next Table).

Therefore, subsequent analyses tested the effect of time in both sex, at all doses, and revealed a dose-dependent increase in methemoglobinemia (Part C of next Table).

At terminal kill, a time-sequenced analyse (Part D of next Table) was performed less than 30 min after exposure, 4 and 19 h after exposure in rats. 19-h after exposure, methemoglobinemia was similar in control, 100 and 350 ppm groups. The level was however significantly increased at 1000 ppm.

Table 102: Methemoglobinemia

Males					**Females**			
0	100	350	1000	Dose levels	0	10	350	1000
A : 15 hours after the 20th exposure								
5	5	5	5	Nb	5	5	5	5
0.8±0.6	0.9±0.3	0.6±0.5	0.6±0.4	MetHb	0.5±0.4	1.0±0.2	0.6±0.5	0.6±0.4
B: immediately after the 29th exposure, in females only								
-	-	-	-	Nb	5	-	-	5
-	-	-	-	MetHb	0.6±0.5	-	-	57.4*±5.2
C: immediately after the 30th exposure								
5	5	5	5	Nb	5	5	5	5
0.6±0.2	2.3±0.2	10.7*±2.2	39.8*±3.9	MetHb	0.4±0.3	4.7*±0.5	26.9*±2.4	70.5*±4.3
D: immediately after the 64th (last) exposure (D92)								
5	5	5	5	Nb	5	5	5	5
0.4±0.4	2.4±0.5	12.9*±1.5	50.7*±5.4	MetHb	0.5±0.3	5.3±1.7	30.7*±3.9	61.8*±6.0
D: 4h after last exposure								
Not det.	Not det.	Not det.	58.6±6.1	MetHb	Not det.	Not det.	Not det.	64.1±4.6
D: 19h after last exposure								
0.5±0.3	0.4±0.3	0.6±0.2	1.5*±0.8	MetHb	0.5±0.3	0.8±0.8	0.8±0.5	1.9*±0.3

MetHb= Methemoglobin level (%), not Det= not determined at this dose level

- *Clinical biochemistry findings:*
  - *Prior to the interim kill (30 days):*

    Alkaline phosphatase: 116±9, 120±5, 115±5 and 108±6 at 0, 100, 350 and 1000 ppm, respectively in males. 98±3, 98±8, 97±10 and 105±8 mU/mL in females, at the same dose level, respectively.

    Glucose: 128±16, 131±21, 120±12 and 123±10 at 0, 100, 350 and 1000 ppm, respectively in males. 101±8, 112*±5, 98±5 and 96±8 mg/100mL in females, at the same dose level, respectively.

Bilirubin: no effect in males, 0.3±0.1, 0.2±0.1, 0.2±0.0 and 0.3±0.0 mg/100mL in females

- *Prior to the terminal kills (92 days):*

  Alkaline phosphatase: 72±7, 70±7, 69±7 and 73±7 at 0, 100, 350 and 1000 ppm, respectively in males. 53±4, 57±7, 58±7 and 67*±6 mU/mL in females, at the same dose level, respectively.

  Glucose: 169±18, 153±16, 147*±16 and 146*±10 at 0, 100, 350 and 1000 ppm, respectively in males. 142±14, 132±10, 136±10 and 117±7* mg/100mL in females, at the same dose level, respectively.

  Bilirubin: no effect in males, 0.3±0.1, 0.2±0.1, 0.3±0.1 and 0.4±0.2 mg/100mL in females

- *Gross pathology findings:*
  - No treatment-related effect on absolute or relative organ weights
  - *Interim kill:* No lesion in the heart, brain, pituitary gland, spinal cord, peripheral nerve, pancreas, bone, adrenal, kidney, small intestine, cecum, male reproductive organs, ovary, oviduct, cervix, vagina, salivary glands, thymus, (para)thyroid, trachea, mammary glands, oral cavity, nasal turbinates, lymph nodes, thoracic cavity, vasculature, aorta, esophagus, lacrimal gland, larynx, colon, rectum.
  - *Terminal kill* (92 days) : no lesion in the heart, brain, pituitary gland, spinal cord, peripheral nerve, pancreas, bone, adrenal gland, stomach, small intestine, cecum, male reproductive organs, ureter, urethra, oviduct, cervix, vagina, skeletal muscle, salivary gland, aorta, esophagus, thyroid and parathyroid glands, trachea, mammary gland, tongue, oral cavity, nasal turbinates, lacrimal gland, larynx, colon, lymph node, rectum, thoracid cavity, vasculature.

Table 103: Macroscopic observations

	Males				**Females**			
Dose levels (ppm)	0	100	350	1000	0	100	350	1000
At interim sacrifice (D 30)								
Nb	5	5	5	5	5	5	5	5
Liver: focal pale right lobe	0	0	0	1	0	0	0	0
Liver: left middle lobe hernia	0	0	0	0	1	0	1	0
Spleen: increased size	0	0	2	5	0	0	0	2
Spleen darkness	0	0	5	5	0	0	0	4
Stomach: multifocal erosion of the glandular mucosa	0	0	0	0	0	0	0	1
Stomach: thickened wall	0	0	0	0	0	0	0	2
Uterus: distened, clear fluids	-	-	-	-	0	1	0	0
Lungs: focal pale lobe	0	1	0	0	0	0	0	0
Eye: cloudy left cornea	0	1	0	0	0	0	0	0

Abdominal cavity: decreased fat	0	0	0	4	0	0	0	4
Perineal soiled aspect	0	0	0	0	0	0	0	3
At terminal kill								
Nb	10	10	10	10	10	10	10	10
Liver: hernia	0	0	0	0	3	2	0	0
Spleen: enlarged	0	0	0	9	0	0	0	10
Spleen: slightly enlarged	0	0	0	1	0	0	0	0
Spleen darkness	0	0	9	10	0	0	0	10
Kidney: bi-lateral darkness	0	0	0	2	0	0	0	0
Distended bladder	0	0	0	1	0	0	0	0
Ovary: right cyst	-	-	-	-	1	0	0	0
Ovary: left cyst	-	-	-	-	0	0	1	0
Uterus: slightly distended, clear fluid	-	-	-	-	0	1	1	0
Lungs: dark left lobe, focal	0	0	1	0	0	0	0	1
Lungs: dark left lobe, multifoc.	0	0	0	0	0	0	0	1
Thymus: slightly decreased	0	0	0	3	0	0	0	0
Eye: decreased right eye	0	0	1	1	0	0	0	0
Decreased left eye	0	0	0	0	0	0	0	1
Multifoc. Haemorr. Right cornea	0	0	0	1	0	0	0	0
Intraocular hemorr. Right eye	0	0	0	0	0	1	0	0
increased vasc. Right cornea	0	0	0	0	0	1	0	0
Right cloudy cornea	0	0	0	1	0	0	0	0
Left cloudy cornea	0	0	0	0	0	0	1	0
Right lens opacity	0	0	0	1	0	1	0	0
Abdomen: strangulated or necrotic fat, omentum	0	0	0	0	1	0	0	0
Decreased fat	0	0	0	3	0	0	0	0
Perineal soiling	0	0	0	0	0	0	0	2

- *Histopathology findings:*
  - *at interim kill:* No lesion in the brain, pituitary gland, spinal cord, peripheral nerve, pancreas, bone, bone marrow, small intestine, mesenteric lymph node, male reproductive organs, urinary bladder, ovary, cervix, oviduct, uterus, skeletal muscle, thymus, aorta, esophagus, para- and thyroid glands, trachea, skin, eye, tongue, mesenteric tissue.
  - *at terminal kill:* No lesion in the brain, spinal cord, peripheral nerve, pancreas, bone, bone marrow, adrenal glands, small intestine, epididymis, seminal vesicle, coagulating gland, prostate, urinary bladder, ovary, oviduct, cervix, uterus, skeletal muscle, thymus, aorta, esophagus, parathyroid gland, skin.

Table 64: Histopathological assessment

	Males				Females			
Dose levels (ppm)	0	100	350	1000	0	100	350	1000
At interim sacrifice (D 30)								
Nb	5	5	5	5	5	5	5	5
With N tissues examined	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Liver : slight mononuclear cells aggregates	1	2	1	1	1	1	1	1
Slight mononucl. aggreg. In the portal area	0	1	1	0	0	0	0	0
Slight focal extramedullary hematopoiesis	0	0	1	0	0	0	0	0
Focal granulomatous inflammation	0	0	0	1	0	0	0	0
Focal necrosis	0	0	0	1	0	0	0	0
Slight diffuse vacuolization	0	0	0	3	5	4	5	5
hernia	0	0	0	0	1	0	1	0
Heart : slight focal infla. myocardium	0	3	0	0	0	0	0	0
Slight multifocal infla. myocardium	0	0	0	0	0	1	0	0
Slight Focal subacute infla.	1	0	0	0	0	0	0	0
Slight Focal subacute myocardial infla.	1	0	0	1	0	0	0	0
Spleen : congestion	0	0	5	5	5	5	5	0
Extramedullary hematopoiesis	0	0	2	5	0	0	0	3
Kidney : decreased tubules cytop. granularity	0	0	0	2	0	0	0	0
Slight focal cortical basophilia	0	0	0	0	1	0	1	0
Slight subacute focal interstitium: inflam.	0	0	0	0	0	1	0	0
Slight focal mineralization CJ	0	0	0	0	0	1	2	0
Slight multifoc. Mineralization CJ	0	0	0	0	2	2	0	0
Lungs: slight multifoc. Mononucl. Aggreg: peribroncholar area	5	5	5	5	5	5	5	5
Slight focal mononucl. Aggreg. Subpleural area	0	1	1	0	0	1	0	1
Slight multifoc mononucl. Aggreg. Subpleural area	0	0	0	1	0	0	1	0
Slight focal mononucl. aggreg. Blood vessels	0	1	1	0	0	0	0	0
Slight Focal subacute inflam. subpleural area	0	0	1	0	0	0	0	0

Nasal turbinates : slight focal mononucl. Aggregates submucosa area	0	0	0	1	3	0	0	0
Slight multifocal mononucl. Aggreg. Submucosa area	5	5	5	4	2	5	4	4
Slight focal degeneration, olfactory epith.	0	0	0	0	0	0	3	0
Slight multifoc. Degen, olfactory epith.	0	0	2	0	0	0	0	0
Slight diffuse degeneration, olf. Epith.	0	0	3	5	0	0	0	5
Slight chronic active inflam. Olf. epithelium	0	0	5	5	0	1	1	5
With N tissues examined	**5**	**0**	**0**	**5**	**5**	**0**	**0**	**5**
Adrenal : slight extramed. hemotopoiesis	0	-	-	0	1	-	-	0
Stomach: diffuse nongland. Submuc. edema	0	-	-	0	0	-	-	1
Diffuse submucosa edema	0	-	-	0	0	-	-	1
Cecum: parasites: nematode	1	-	-	0	0	-	-	0
Large intestine: parasites: nematode	0	-	-	1	0	-	-	1
Cervical lymph nodes: erythrophagocytosis	0	-	-	0	0	-	-	1
Salivary gland: slight acini vacuolization	5	-	-	5	0	-	-	0
Mammary gland: N tissues examined	4	0	0	4	5	-	-	5
Slight acini hyperplasia	4	-	-	4	0	-	-	0
Slight ducts hyperplasia	0	-	-	0	5	-	-	5
At terminal kill								
Nb	5	5	5	5	5	5	5	5
With N tissues examined	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Liver: slight focal aggregates of mononuclear cells	0	0	0	0	0	0	0	1
Diaphragmatic hernia causing altered architecture	0	0	0	0	2	0	0	0
Very slight mutifoc extramed. Hematopoiesis	2	0	0	1	0	0	0	0
Slight multifocal extramed. Hematopoiesis	0	0	0	0	0	0	0	1
Subcapsular fibrosis	0	0	0	0	0	0	0	1
Focal subcapsular fibrosis	0	0	0	0	1	0	0	0
Subcapsular hematogenous pigment	0	0	0	0	0	0	0	1
Very slight multifoc. Vacuolization	2	0	0	0	0	0	0	0
Slight multifocal vacuolization	0	0	2	5	0	0	0	3

Slight diffuse vacuolization	0	0	0	0	0	1	4	0
Heart: slight focal subacute inflame. myocardium	0	1	0	0	0	0	0	0
Slight multifoc. subacute inflame. myocardium	0	0	1	0	0	0	0	0
Slight multifocal necrosis	0	0	0	0	0	0	0	1
Spleen: congestion	0	5	5	5	0	5	4	5
Extramed. Hematopoiesis	0	5	5	5	0	1	2	1
Slight extramed. Hematopoiesis	0	0	0	0	0	0	1	0
Slight increased hematogenous pigmentation	0	0	0	0	0	0	1	0
Slight increased hematogenous pigmentation red pulp	0	0	0	0	0	0	0	1
Pituitary gland: anterior cyst	0	0	0	0	0	1	0	0
Pars intermedia cyst	0	0	0	1	0	0	0	0
Kidney : slight focal mononuclear aggregates in the cortical area	0	0	0	1	0	0	0	0
Slight focal mononucl aggregates, unilat, pelvis area	0	0	0	1	0	0	1	0
Decreased bilateral cortical cytop. Granularity	0	0	0	5	0	0	0	0
Slight focal unilateral cortical fibrosis	0	0	0	1	0	0	0	0
Slight focal unilateral cortical basophilia	1	0	1	1	0	1	0	0
Slight multifoc unilat cortical basophilia	2	1	1	0	0	0	0	0
Slight multifocal unilat mineralization of CJ	1	0	0	0	1	1	0	0
Slight multifoc bilat mineralization CJ	0	0	0	0	1	3	1	2
Stomach: N tissues examined	5	5	5	4	5	5	5	5
Slight focal mononucl. Aggreg. submucosa	1	1	0	0	0	0	0	0
Cecum : N tissues examined	5	5	5	2	5	4	5	4
Nematodes – parasites:	1	1	0	0	1	1	0	0
Large intestine: N tissues examined	5	5	4	4	5	5	5	3
Parasites: nematodes	0	3	0	0	0	0	0	0
Testes: slight decreased spermatogenesis (/5)	0	1	0	0	-	-	-	-
Lungs : N tissues examined	5	5	5	5	5	5	5	5
Slight multifocal mononucl aggreg. Peribronchiolar area	5	5	5	5	5	5	5	5
Slight focal mononucl. Aggreg.	1	0	0	1	1	0	0	0

Subpleural area								
Slight focal subpleural fibrosis	1	0	0	0	0	0	0	0
Slight multifocal haemorrhage	0	0	0	0	0	0	0	2
Slight multifocal acute inflammation	0	0	0	0	0	0	0	2
Slight focal subacute inflammation	0	0	0	0	0	0	0	1
Slight focal pigment-laden macrophages	0	0	0	0	0	0	0	1
Slight multifocal pigment-laden macrophages	0	0	0	0	0	0	0	2
Slight multifoc lymphoid perivascular cuffing	0	0	0	0	0	0	1	1
Salivary gland: N tissues examined	5	5	5	5	5	5	5	5
Very slight ductal decreased cytop. granularity	0	5	0	0	0	5	0	0
Slight decrease in ductal cytop. granularity	0	0	5	5	0	0	5	5
Very slight decreased ductal eosinophilia	0	5	0	0	0	5	0	0
Slight decreased ductal eosinophilia	0	0	5	5	0	0	5	5
Acini vacuolization	0	0	0	0	0	0	0	3
Trachea : N tissues examined	5	5	5	5	5	5	5	5
Slight focal mononucl aggreg. Submucosa	0	2	2	0	2	1	0	0
Mammary gland : N tissues examined	2	3	1	1	4	3	5	5
Slight acini hyperplasia	1	1	1	1	0	1	0	0
Slight ductal hyperplasia	0	0	0	0	0	0	1	1
Eye : N tissues examined	5	5	4	5	5	5	5	5
Decreased size	0	0	1	0	0	0	0	0
Fibrosis	0	0	1	0	0	0	0	0
Fibrosis, posterior chamber area	0	0	0	1	0	0	0	0
Haemorrhage	0	0	0	0	0	1	0	0
Unilateral haemorrhage	0	0	0	0	0	1	0	0
Unilateral hematogenous pigment	0	0	0	0	0	1	0	0
Osterior chamber hematogenous pigment	0	0	0	1	0	0	0	0
Nasal turbinates: N tissues examined	5	5	5	5	5	5	5	5
Slight multifoc mononucl aggreg, submucosa	5	5	5	5	5	5	5	5
Slight focal degeneration olfactory epith	0	0	1	0	0	0	0	0
Slight diffuse degen. Olf. Epith.	0	0	1	0	0	0	2	0
Moderate diffuse degen. Olf. Epith.	0	0	0	5	0	0	0	5
Moderate multifoc. degen. Respiratory epith.	0	0	0	1	0	0	0	0

Slight acute inflammation Resp. epith	0	0	1	0	0	0	0	0
Slight multifocal acute infla. Vomeronasal organ	0	1	0	0	0	0	0	0
Slight focal chronic active inflame. Olf. epith	0	0	1	0	0	0	0	0
Slight multifocal Chronic Active inflammation Olfactory epithelium	0	0	1	0	0	0	0	0
Slight diffuse chronic active inflame. Olf. epith	0	0	0	4	0	0	2	5
Moderate diffuse chronic active inflame. Olf. epith	0	0	0	1	0	0	0	0
Slight diffuse subacute inflammation of respiratory epithelium	0	0	0	1	0	0	0	0
Slight focal metaplasia of rep. epith.	1	0	0	0	0	0	0	0

CJ= corticomedullary junction

3.12.1.2.2 13-week repeated dose inhalation toxicity study in mouse (Anonymous 26, 1982)

Study reference:

Anonymous 26, 1982

Detailed study summary and results: The subchronic toxicity of nitroethane was examined in mice. Groups of mice were exposed to 0, 100, 350 or 1000 ppm (0, 0.3, 1.0 or 3.0 mg/L) of nitroethane for 6 h/d, 5 d/week for a total of a 93-d period and an interim sacrifice of rats after a 29-d period. Parameters monitored were clinical observations, body weights, organ weigths, hematological characteristics including methemoglobin (MetHb) determination, clinical chemistries, gross pathology and histopathology. The LOAEC was determined at 100 ppm for males based on systemic effects on MetHb and liver after 13 weeks exposure.

Test type

- Equivalent or similar to OECD TG 413 (Major deviation : feed consumption was not measured)
- GLP-compliant according to the registration dossier, but Study was initiated prior to GLP and completed with GLP
- Reliability 1 (according to the registration dossier)

Test substance

- Nitroethane
- *Degree of purity:* 97 %
- *Impurities:* Nitromethane < 1 %; 2-Nitropropane < 1.5 %

Test animals

- *Species/strain/sex:* mouse / B6C3F1 / both sexes
- *Nb. of animals per sex per dose:* 10/sex/dose + interim group of 5/sex/dose.

[NOTE : As a result of early mortalities in the mice, 5 or less mice/sex/dose were necropsied at the interim kill (29 days), to insure that at least 10 mice/sex/dose would continue exposure to the terminal kill. Also, at the terminal kill, it was discovered that 2 mice originally assigned to the study as males in the 350 ppm group were found to be female, giving 8 males and 12 females for this group.]

- *Age and weight at the study initiation :* 7 weeks old, weight not specified

Administration/exposure

- *Route of administration :* inhalation (vapours)
- *Duration and frequency of test/exposure period :* 6 h/d, 5 d/weeks (excluding holidays) for 29 days (5/sex/dose, interim group) or for 93 days (10/sex/dose)
- *Doses/concentration levels, rationale for dose level selection :* 0, 100, 350 or 1000 ppm (corresponding to 0, 0.3, 1.0 or 3.0 mg/L)
- *Post exposure observation period:* /
- *Vehicle:* air
- *Control group and treatment:* Sham-treated animals
- *Statistical methods :* Analysis of variance and Dunnett's test using a level of significance of $p<0.05$
- *Type of inhalation exposure and test conditions (e.g.: exposure apparatus):* 1 cubic meter stainless steel and glass Rochester-type chamber under dynamic airflow conditions. (Airflow 175 i/min, Temperature 70 °F, relative humidity 50 %)
- *Method of exposure ("whole body", "oro-nasal", or "head only"), exposure data:* whole body
- *Analytical verification of test atmosphere concentrations:* Infrared spectrophotometer equipped with a variable pathlength gas cell. Wavelength 11.5 microns. Analysis performed 1-2 times per hour for each exposure concentration.

Results and discussion

Mice were exposed during 13 weeks to 0, 100, 350 or 1000 ppm nitroethane. The results obtained show an increased methemoglobinemia, effects in the salivary glands, liver, olfactory nasal epithelium and multinucleated spermatids in the testes at 1000 ppm. At 350 ppm, methemoglobinemia, effects in the liver, salivary glands and nasal epithelium were seen. At the lowest dose, minimal effects were reported in the nasal epithelium, and transient effects on the epithelium of the salivary glands.

- *Mortality and time to death:*

Table 104: Mortality reported during the study and at interim and terminal kills

	Nb at start		**Spontaneous death**		**Interim kill**		**Terminal kill**	
Dose level	Male	female	male	female	male	female	male	female
0 ppm	15	15	1	0	5	5	9	10
100 ppm	15	15	0	0	5	5	10	10
350 ppm	13	17	2	0	3	5	8	12

1000 ppm	15	15	1	0	4	5	10	10

- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* no clinical signs are mentioned in the study report for mice.
- *Body weight and body weight changes:* no effects
- *Food/water consumption:* not measured
- *Sensory activity, grip strength and motor activity assessments (when available):* /
- *Ophthalmologic findings: incidence and severity*
- *Haematological findings:* The statistically significant changes found in the PCV, RBC and Hb parameters at the interim and terminal analysis were within the normal variability for the B6C3F1 mouse. Increased reticulocytes and Heinz bodies were detected in the mice of the 350 and 1000 ppm groups at the interim and terminal kills.

Table 105: Hematological data

Males				**Exposure**	Females			
0	100	350	1000	**(ppm)**	0	100	350	1000
At interim kill								
46.7±1.7	47.3±1.2	48.7±1.3	51.0*±0.7	PCV	47.2±0.6	47.6±1.1	48.3±3.0	47.1±1.9
8.70±0.20	9.09±0.19	8.93±0.43	9.17*±0.21	RBC	8.89±0.58	8.94±0.28	9.14±0.26	8.57±0.31
14.6±0.3	15.4±0.4	15.1±0.7	15.9*±0.3	Hb	15.3±0.9	15.3±0.6	15.8±0.4	15.1±0.4
4.0±1.6	3.5±0.8	2.4±1.3	4.6±0.9	WBC	2.0±0.7	3.4±0.7	2.8±1.1	3.8*±1.1
1.1±0.3	1.3±0.2	1.4±0.2	1.0±0.1	Ret.	0.6±0.4	1.0±0.2	1.2*±0.4	1.1*±0.3
0.6±0.2	0.8±0.3	2.1*±0.1	5.9*±0.5	Heinz bodies	0.6±0.1	0.5±0.0	1.2±0.2	7.3*±1.3
At terminal kill								
43.6±3.4	44.1±1.8	44.0±1.2	44.1±3.4	PCV	44.5±1.7	45.1±1.9	45.2±2.2	48.7*±1.7
8.65±0.84	8.86±0.26	8.87±0.50	7.86±0.61	RBC	8.93±0.46	8.63±0.30	8.41*±0.11	8.65±0.2
14.3±1.0	14.2±0.4	14.4±0.4	14.0±0.9	Hb	14.6±0.7	14.2±0.5	14.2±0.4	15.0±0.6
3.7±1.0	3.8±0.9	4.9±0.9	3.8±1.1	WBC	3.3±1.5	1.9±0.7	2.4±0.8	2.3±0.4
1.6±0.7	1.4±0.7	2.1±0.3	3.5±2.4	Ret.	0.7±0.3	1.2±1.2	1.5*±0.8	1.8*±0.4
1.8±1.1	3.3±1.5	5.2±4.3	10.7*±7.6	Heinz bodies	0.6±0.2	1.3±0.2	1.8±0.6	8.6*±3.4

PCV= packed cells volume (%); RBC= Red blood cells ($x10^6/mm^3$); Hb= Hemoglobin (g/100mL); WBC= White blood cells ($x10^3/mm^3$); Reticulocytes (%); Heinz bodies (%)

- *Methemoglobinemia*: At terminal kill, a time-sequenced analyse of methemoglobinemia levels was performed less than 30 min after exposure, 4 and 19 h after exposure in mice. 19-

h after exposure, methemoglobinemia was similar in control, 100 and 350 ppm groups and in males exposed to 1000 ppm. The level was however significantly increased at 1000 ppm, in females.

Table 106: Methemoglobinemia

Males					Females			
0	100	350	1000	Dose levels	0	100	350	1000
5	5	5	5	Nb	5	5	5	5
Immediately after the 64th (last) exposure (D 92)								
0.8±0.3	1.2±0.4	6.6*±4.3	36.4*±3.0	MetHb	1.2±0.7	0.9±0.7	5.8*±1.8	20.8*±2.0
4 h after last exposure								
Not det.	Not det.	Not det.	7.4±2.6	MetHb	Not det.	Not det.	Not det.	10.4±2.9
19 h after last exposure								
0.8±0.7	0.8±0.4	1.3±1.0	0.9±0.4	MetHb	1.1±0.3	0.9±0.6	1.3±0.4	2.4*±0.8

MetHb= Methemoglobin level (%), not Det= not determined at this dose level

- *Clinical biochemistry findings:*
  - *Prior to the interim kill (30 days)*: no effects were seen on SGPT (serum glutamic-pyruvic transaminase) and calcium blood levels of males and females

Table 107: Clinical biochemistry parameters at interim sacrifice

Males				**Exposure (ppm)**	Females			
0	100	350	1000		0	100	350	1000
36±5	28±6	29±9	20*±2	**BUN**	30±7	17*±3	21*±6	16*±3
55±9	54±4	55±8	48±5	**ALP**	85±4	71*±7	75±13	65*±5
8.5±1.3	8.6±0.5	7.9±1.2	7.2±2.0	**P**	10.9±0.5	10.7±1.4	10.4±1.7	7.6*±0.6

BUN = blood urea nitrogen (mg/100 mL); ALP= alkaline phosphatase (mU/mL); P= phosphorus (mg/100 mL)

  - *Prior to the terminal kills (92 days):* no effects were seen on SGPT, ALP, glucose, phosphorus and calcium levels on on mice from which blood was already punctured the day before to assess MetHb. No changes was reported in SGPT, ALP, glucose, and phosphorus blood levels at terminal kill, in mice never bled before.

Table 108: Clinical biochemistry parameters at terminal kill

Males				**Exposure (ppm)**	Females			
0	100	350	1000		0	100	350	1000
At terminal kill, on mice from which blood was already punctured the day before to assess MetHb								
38±6	36±10	44±12	30±4	**BUN**	29±3	21*±2	25±4	33±5
39±6	46±7	43±7	37±2	**ALP**	59±7	58±7	53±15	49±7
8.2±0.6	9.4±0.5	9.6±0.6	8.8±2.1	**P**	8.9±1.1	7.5±0.7	6.9±2.1	8.4±1.0

At terminal kill, on mice never bled before								
34±5	29±2	20*±2	27±6	**BUN**	26±4	21±3	19*±2	20*±3
45±6	36±5	38±4	39±7	**ALP**	54±8	60±7	55±6	63±12
10.7±2.0	8.3±0.3	9.3±1.9	9.4±1.0	**P**	8. 2±0.6	7.3±1.2	8.0±0.9	8.4±1.1
10.5±0.6	11.2±0.8	9.9±0.3	10.0±0.2	**Ca**	10.2±0.2	10.0±0.5	9.8±0.2	9.6*±0.1

BUN = blood urea nitrogen (mg/100 mL); AP= alkaline phosphatase (mU/mL); P= phosphorus (mg/100 mL); Ca=Calcium (mg/100 mL)

- *Gross pathology findings:*
  - *At interim kill:* no macroscopic lesions were seen in males and females, except for alopecia in the thoracic area of 1/3 males exposed to 350 ppm.
  - *At terminal kill:* no gross findings were reported except for:
    - At 100 ppm: severe unilateral decrease in the size of a testicle and epidydimis in 1/10 males, unilateral preputial abscess in 1/10 males, and moderate alopecia on the abdomen and thorax on 1/10 females.
    - At 350 ppm: a slightly increased spleen in 1/8 males, and one focal preputial ulcer was reported in 1/8 males.
    - At 1000 ppm, an ovary nodule in 1/10 females
- *Organ weight:*
  - *Prior to the interim kill (30 days):* No changes in absolute liver, kidney, and brain weights in both sex. No changes in absolute heart weights, nor in absolute and relative thymus and testes weights in males. In females, heart absolute weights were slightly decreased in all treatment groups (0.13±0.01, 0.11*±0.01, 0.10*±0.01 and 0.10*±0.01 at 0, 100, 350 and 1000 ppm, resp.). Mean relative heart weigths in females were only significantly decreased at the highest dose level (0.50±0.04, 0.45±0.05, 0.45±0.03 and 0.42*±0.04 at 0, 100, 350 and 1000 ppm, resp.).

    No changes in kidney relative weights, in females.
  - *Prior to the terminal kills (92 days):* No treatment related effect on liver absolute and relative weights, in both sex. Kidney, heart and brain relative and absolute weights were not affected by the treatment in males. Testes relative weights were significantly increased at mid and high doses. In females, kidneys relative weights were significantly increased at low and mid doses; while heart relative weights were significantly decreased at mid and high dose levels. Brain absolute and relative weights were significantly decreased at high dose level, in females. Thymus weights were not affected, in females.

Table 109: organ weight data

	Males				**Females**			
Dose levels (ppm)	0	100	350	1000	0	100	350	1000
At interim kill								

Nb	5	5	3	4	5	5	5	5
Mean BW	27.4±0.9	28.4±2.5	28.3±1.5	27.3±1.7	26.2±1.3	24.0±0.7	23.4±2.7	23.8±1.6
Liver (rel) (%)	6.08±0.26	5.64±0.21	5.20*±0.24	6.06±0.3	5.45±0.21	5.40±0.34	5.44±0.26	6.36*±0.25
Kidney (rel) (%)	2.04±0.13	1.75*±0.11	1.72*±0.2	1.76*±0.11	No changes			
Thymus (abs) (g)	No changes				0.06±0.01	0.04*±0.00	0.03*±0.01	0.02*±0.01
Thymus (rel) (%)	No changes				0.23±0.03	0.18*±0.02	0.14*±0.05	0.10*±0.02
At terminal kill								
Mean BW	34.3±2.0	33.6±2.5	32.4±2.6	32.4±2.5	27.4±1.8	28.1±1.4	27.7±1.4	28.4±1.6
Kidney (rel) (%)	No changes				1.38±0.11	1.47*±0.04	1.49*±0.06	1.42±0.1
Heart (rel) (%)	No changes				0.49±0.06	0.49±0.05	0.42*±0.03	0.41*±0.03
Brain (abs) (g)	No changes				0.46±0.02	0.47±0.02	0.45±0.02	0.43*±0.02
Brain (rel) (%)	No changes				1.69±0.12	1.66±0.08	1.63±0.05	1.53*±0.09
Thymus (abs) (g)	0.04±0.01	0.03±0.01	0.03±0.01	0.02*±0.01	No changes			
Thymus (rel) (%)	0.11±0.03	0.09±0.04	0.08±0.02	0.08*±0.03	No changes			
Testes (abs) (g)	0.22±0.02	0.22±0.02	0.23±0.02	0.23±0.02	N/A			
Testes (rel) (%)	0.64±0.06	0.65±0.05	0.70*±0.05	0.72*±0.03	N/A			

N/A: not applicable

- *Histopathology findings:*
  - *Prior to the interim kill (30 days):* 1000 ppm group : Hepatocellular vacuolization consistent with fatty change in females. At interim kill, no modifications in gall bladder, heart, spleen, brain, pituitary gland, peripheral nerve, pancreas, bone, bone marrow, adrenal gland, kidney, small intestine, cecum, large instestine, lymph nodes, seminal vesicles, coagulating gland, urinary bladder, ovary, oviduct, uterus, cervix, skeletal muscle, esophagus, para- and thyroid glands, trachea, skin, mammary gland, eye.

    Slight focal glandular granuloma in the stomach submucosa and slight focal chronic active submucosal inflammation were seen in 1/4 control male, however, it is not mentioned if it was the same animal that was affected. Dermoid cyst in meninges and ectopic thymic tissue was reported in 1/4 control female, however, it is not specified if it was the same animal affected.
  - *At terminal sacrifice (92 days)*: no effects were reported on the gallbladder, spleen, brain, pituitary – salivary – mammary glands, thyroid – parathyroid, peripheral nerve, pancreas, bone, bone marrow, stomach, small intestine, cecum, large intestine, lymph nodes, thymus, esophagus, trachea, skin, eye, epididymis, seminal vesicle, coagulating gland, prostate, urinary bladder, oviduct, uterus, lungs, skeletal muscle.

    Slight multifocal mineralization of the myocardium was reported in 1/5 control male. Focal dermoid cysts in spinal cord meninges was seen in 1/5 control female. Multifocal mononuclear cells aggregates were seen in 2/5 control females.

Table 110: Histopathological modifications

	Males				Females			
Dose levels (ppm)	0	100	350	1000	0	100	350	1000
At interim sacrifice								
N	5	5	5	5	5	5	5	5
Liver : Nb tissues examined	5	5	3	4	5	5	5	5
Slight focal mononucl aggreg.	0	0	0	0	1	0	0	0
Slight multifocal mononucl. aggreg.	0	0	0	0	1	1	1	0
Slight focal mononucl. aggreg. portal area	0	0	0	0	1	0	0	0
Altered cells tinctorial properties	0	0	0	0	0	0	1	0
Diffuse hepatocellular vacuolization	0	0	0	4	0	0	1	5
Testicles: N tissues examined:	5	0	0	4	-	-	-	-
Slight focal unilateral decreased spermatogenesis in tubules	0	0	0	1	-	-	-	-
Slight focal unilateral interstitial hyperplasia	0	0	0	1	-	-	-	-
Epididymis : Nb tisssues examined:	5	0	0	4	-	-	-	-
Slight focal mononuclear aggregates	0	0	0	1	-	-	-	-
Prostate : Nb tissues examined	3	0	0	3	-	-	-	-
Slight focal mononuclear aggregates	2	0	0	3	-	-	-	-
Lungs : Nb tissues examined	5	5	3	4	5	5	5	5
Slight multifoc peribronch. mononuclear aggregates	0	0	0	0	0	1	0	0
Salivary gland: Nb tissues examined	5	0	0	4	5	5	5	5
Very slight decrease in ductal. C.G.	0	0	0	0	0	1	0	0
Slight decrease in ductal C.G.	0	0	0	0	0	4	0	1
Moderate decrease in ductal C.G.	0	0	0	0	0	0	5	4
Very slight decrease in eosinophilia	0	0	0	0	0	1	0	0
Slight decrease in eosinophilia	0	0	0	0	0	4	0	1
Moderate decrease in eosinophelia	0	0	0	0	0	0	5	4
Mediastinal tissue: Nb tissues examined	5	4	2	4	3	5	2	5
Multifocal mononcl.aggregates	0	0	0	0	0	0	0	1
Slight multifoc. Mononucl. aggregates	2	3	2	2	4	3	2	3
Nasal turbinates: Nb tissues examined	5	5	3	4	5	5	5	5
Slight multifocal mononuclear aggregates	0	0	0	0	0	1	0	0
Slight multifoc. Submucosa mononuclear aggregates	4	5	3	4	2	4	5	5
Slight olf. epith degeneration ± inflam	0	0	0	0	0	0	1	0
Moderate olf. epith degeneration ± inflam	0	0	3	4	0	0	4	5
Slight glandular hyperplasia olfactory epith	0	0	0	0	0	0	0	1
Moderate glandular hyperplasia olf. epith	0	0	2	4	0	0	4	4
Mesenteric tissue: Nb tissues examined	5	1	0	4	5	0	0	5

Slight multifocal mononuclear aggregates	1	1	0	0	2	0	0	0
At terminal kill								
Liver : Nb tissues examined	5	5	5	5	5	5	5	5
Very slight focal mononuclear aggregates	0	0	0	0	0	0	0	1
Very slight focal mononuclear aggregates next to degenerative or necrotic cells	0	0	0	1	0	1	1	0
Slight increase in centrilobular cytoplasmic homogenity	0	0	3	5	0	0	2	5
Slight focal vacuolated or clear cells	0	0	0	0	0	0	1	0
Adrenal : Nb tissues examined	5	0	0	5	5	0	0	5
Very slight focal unilat. hyperplasia (spindle cells, Z.G.)	0	0	0	1	0	0	0	0
Very slight multifoc. bilat. hyperplasia (spindle cells, Z.G.)	0	0	0	1	2	0	0	4
Slight multifocal bilateral hyperplasia (spindle cells, Z.G.)	0	0	0	0	2	0	0	0
Kidney : Nb tissues examined	5	5	5	5	5	5	5	5
Very slight focal unilateral C.J. mononucl. aggregates	0	1	0	0	0	0	0	0
Very slight focal unilat. Interstitial mononucl. Aggregates	1	0	0	0	0	0	0	0
Very slight focal unilat. Pelvic epithelium mononucl. Aggreg	1	0	0	0	0	0	0	0
Slight focal unilateral basophilic cortex	1	0	0	0	0	0	0	0
Mediastinal tissue : Nb tissues examined	5	0	0	5	5	0	0	5
Slight multifocal mononuclear aggregates	2	0	0	0	0	0	0	2
Tongue : Nb tissues examined	5	0	0	5	5	0	0	5
Very slight focal submucosa subacute inflammation	0	0	0	1	1	0	0	0
Nasal turbinates: Nb tissues examined	5	5	5	5	5	5	5	5
Slight focal abscess	1	0	0	0	0	0	0	0
Slight multifoc submucosa mononuclear aggregates	5	4	3	4	3	5	3	5
Diffuse unilateral degenerated olf. epith.	0	0	0	0	1	0	0	0
Very slight diffuse unilateral degenerated olf. epith.	1	0	0	0	0	0	0	0
Slight diffuse unilat degenerated olf. epith.	2	1	0	0	0	0	0	0
Moderate diffuse unilat degenerated olf. epith.	1	0	0	0	1	0	0	0
Slight olf. epith. degeneration ± inflammation	0	0	1	0	0	0	0	0
Moderate olf. epith. degeneration ± inflammation	0	0	4	5	0	0	5	5
Slight glandular olf. epith. hyperplasia	0	0	0	1	0	1	0	0
Moderate glandular olf. epith. hyperplasia	0	0	4	4	0	0	5	5
Testicles : Nb tissues examined	5	0	0	5	-	-	-	-

Slight fical unilateral fibrinoid degeneration in tubules	1	0	0	0	-	-	-	-
Very slight multifocal bilateral multinucleated spermatids	0	0	0	1	-	-	-	-
Slight multifoc. bilat. multinucleated spermatids	0	0	0	1	-	-	-	-
Very slight multifoc. bilat. multinucl. spermatids in tubules	0	0	0	1	-	-	-	-
Ovary : Nb tissues examined	-	-	-	-	5	0	0	5
Primary benign teratoma, no metastasis	-	-	-	-	0	0	0	1
Cervix : Nb tissues examined	-	-	-	-	4	0	0	5
Very slight focal muscularis acute inflam.	-	-	-	-	0	0	0	1
Lacrimal gland: Nb tissues examined	2	1	2	1	1	0	0	2
Moderate acute inflammation	0	0	0	0	0	0	0	1
Moderate unilateral acute inflammation	1	0	0	1	0	0	0	0
Slight focal unilateral acute inflammation	0	1	1	0	1	0	0	0
Slight multifocal unilateral actue inflammation	0	0	1	0	0	0	0	0
Moderate multifocal unilateral acute inflammation	1	0	0	0	0	0	0	1

C.G.= cytoplasmic granularity; Z.G.= zona glomerula; Olf. Epith. = olfactory epithelium; unilat.= unilateral; bilat.= bilateral

- *Gross pathologic observations in mice dying during experiment:* 1, 0, 2 and 1 male mice died during the experiment in groups exposed to 0, 100, 350 and 1000 ppm nitroethane, respectively. No macroscopic lesions was reported except, at 350 ppm, thymus atrophy in 1/2 male, decreased abdominal fat in 1/2 male, loss of body condition in 1/2 male, and slight soiled perineum in 1/2 male.
- *Histopathologic findings in mice dying during the experiment:* 1, 0, 2 and 1 male mice died during the experiment in groups exposed to 0, 100, 350 and 1000 ppm nitroethane, respectively. 0, 0, 2 and 1 mice were examined for histological assessment. No lesions were found except for:
    - Slight multifocal submucosa mononuclear aggregates in 1/2 males exposed to 350 ppm
    - Moderate degeneration of the olfactory epithelium, without or with inflammation in 2/2 and 1/1 males exposed to 3502 and 1000 ppm, respectively
    - Moderate glandular hyperplasia in the olfactory epithelium in 1/2 and 1/1 males exposed to 350 and 1000 ppm, respectively

3.12.1.2.3 Chronic inhalation toxicity study (Anonymous 35, 1986)

Study reference:

Anonymous 35, 1986

Detailed study summary and results:

Male and female Long-Evans rats were exposed by inhalation to vapors of nitroethane (NE) at either 100 ppm or 200 ppm, seven hours per day, five days per week for two years. General observations were made daily and body weights were obtained weekly or biweekly.

During the study any rats that were found dead or sacrificed moribund were given a thorough gross examination and tissues retained for microscopic examination. After two years of inhalation of NE, all surviving rats were sacrificed. Blood samples were obtained from selected individuals for hematology and serum chemistry studies. All rats were examined histopathologically.

Exposure of the rats to NE had no pharmacologic effects nor were there any effects on mortality of rats in either sex at any level of exposure. Body weights of both sexes of both exposed groups were slightly less than controls, but lack of a well-defined dose-response relationship suggested the involvement of factors other than just exposure to NE. There were no effects of exposure to NE on hematology. There were no biologically significant effects of exposure to NE on clinical chemistry or on organ weights. There was no significant difference in the non-neoplastic or neoplastic pathology related to exposure to NE.

See section 3.9 Carcinogenicity

3.12.1.3 Animal data on 1-NITROPROPANE

3.12.1.3.1 Combined repeated dose toxicity study with reproduction and developmental toxicity screening test (Anonymous 37, 2003)

See section 3.10.1.3.1

3.12.1.3.2 28-day oral repeated dose toxicity study (Anonymous 38, 1996)

Study reference:

Anonymous 38, 1996

Detailed study summary and results:

Test type

- 28-day repeated dose toxicity study
- Japanese guideline
- GLP
- Reliability 1 (according to the registration dossier)

Test substance

- 1-nitropropane
- *Degree of purity:* >98.5 %

Test animals

- *Species/strain/sex:* rat / SD / both sexes
- *Nb. of animals per sex per dose:* 5/sex/dose

- *Age and weight at the study initiation:* 121 to 161g for males and 121 to 159g for females, 5 to 6w old

Administration/exposure

- *Route of administration:* oral (gavage)
- *Duration and frequency of test/exposure period:* 28 days, daily
- *Doses/concentration levels:* 0, 10, 30, 100 mg/kg bw/d + 2 additional groups of 0 and 100 mg/kg bw/d
- *Post exposure observation period :* 14 days for recovery groups
- *Vehicle:* arachis oil

Results and discussion

- *Mortality and time to death (if occurring) :* 1 male exposed to the highest dose was killed in extremis at D 27.
- *Description, severity, time of onset and duration of clinical signs :* increased salivation in animals exposed to 100 mg/kg bw/d was noted.
- *Body weight and body weight changes :* a slight body weight decrease was noted at the highest dose in males. This change was not observed nor in males of the recovery group nor in females.

Table 111: Body weight data (in g)

	Main groups				Recovery groups	
Dose level (in mg/kg bw/d)	0	10	30	100	0	100
Males						
D 0	138	141	143	140	142	141
D 14	249	255	267	236	256	254
D 21	298	305	327	281	302	295
D 28	329	338	368	296	345	334
D 42	/	/	/	/	399	390
Females						
D 0	131	145	137	140	137	143
D 14	199	205	197	197	196	201
D 21	221	229	226	220	220	221
D 28	231	245	239	234	240	236
D 42	/	/	/	/	263	253

- *Sensory activity, grip strength and motor activity assessments (when available) :* not examined
- *Ophthalmologic findings:* not examined

- *Haematological findings:* statistically significant lower hemoglobin, hematocrit values and erythrocyte count and a statistically significant higher clotting time were observed in females of the highest dose, however these values were within the range of the historical control data.

Table 112: Hematological findings

	Males						Females					
	Main groups				Satellite group		Main groups				Satellite group	
Dose level (in mg/kg bw/d)	0	10	30	100	0	100	0	10	30	100	0	100
Hb (g/dL)	14.7	14.9	15.1	14.0	15.6	16.4	14.9	14.3	14.2	14.1*	15.3	14.6
Ht (%)	43.2	43.9	44.2	42.3	44.6	46.4	43.6	42.4	41.6	40.2**	43.5	41.3*
RBC (10^{12}/L)	7.78	7.72	7.72	7.65	8.12	8.48	7.80	7.60	7.48	7.38*	7.88	7.64
WBC (10^9/L)	13.0	12.4	12.6	14.0	12.3	14.4	11.4	9.4	12.3	14.5*	11.9	10.3
Meth (%)	0.87	2.67*	0.94	1.19	0.54	1.12**	0.47	0.54	0.93	1.28	0.34	0.35
Lymph (10^9/L)	11.26	10.17	11.14	12.46	9.24	11.81*	9.35	8.06	10.94	12.67*	8.38	7.37
CT (s)	26	27	27	28	26	26	25	27	27	28*	25	26
Plt (10^9/L)	1102	1174	1220	1115	1304	1080**	1094	1156	1056	1264	1112	1140

- *Clinical biochemistry findings:* no treatment-related effect was observed

Table 113: Clinical biochemistry values

	Males						Females					
	Main groups				Satellite group		Main groups				Satellite group	
Dose level (in mg/kg bw/d)	0	10	30	100	0	100	0	10	30	100	0	100
Urea (mg/dL)	20	25	24	32**	26	26	33	29	36	30	29	26
A/G	1.10	1.15	1.08	1.06	1.30	1.26	1.03	1.06	1.05	1.10*	1.23	1.15
ALP (IU/L)	700	618	641	576	553	524	526	376*	445	378*	297	299
Tri (mg/dL)	93	77	130	115	100	106	41	52	46	67*	63	54

- *Gross pathology findings:* at the highest dose, animal, which was killed in extremis, exhibited dark kidneys, thickening of the forestomach and sloughing of the glandular gastric epithelium
- *Organ weight :*
    - *Males:* FBW: 329, 333, 365 and 292 g resp. at 0, 10, 30 and 100 mg/kg bw/d for main groups and 391 and 385 resp. at 0 and 100 mg/kg bw/d for satellite groups. Animals exposed to 100 mg/kg bw/d (main group) exhibited a significant higher absolute brain weight (1.9961, 2.0477, 1.9955 and 2.0775* g resp. at 0, 10, 30 and 100 mg/kg bw/d in main groups

and 1.9952 and 2.0260 g resp. at 0 and 100 mg/kg bw/d in satellite groups) and a significant lower pituitary weight (0.0091, 0.0102, 0.0103 and 0.0072* g resp. at 0, 10, 30 and 100 mg/kg bw/d in main groups and 0.0105 and 0.0096 g resp. at 0 and 100 mg/kg bw/d in satellite groups). The relative brain weight was also significantly higher (0.6076, 0.6189, 0.5515 and 0.7169g resp. at 0, 10, 30 and 100 mg/kg bw/d in main groups and 0.5126 and 0.5297g resp. at 0 and 100 mg/kg bw/d in satellite groups).

- *Females:* FBW: 231, 243, 235 and 227 g resp. at 0, 10, 30 and 100 mg/kg bw/d in main groups and 259 and 250 g resp. at 0 and 100 mg/kg bw/d in satellite groups. A higher brain weight was noted in animals of the mid and high dose levels (1.8593, 1.8909, 1.9453* and 2.0206*** g resp. at 0, 10, 30 and 100 mg/kg bw/d in main groups and 1.9062 and 1.8947 g resp. at 0 and 100 mg/kg bw/d in satellite groups). Moreover, animals exposed to the highest dose exhibited a significantly higher kidneys weight (1.6071, 1.6922, 1.6761 and 1.7762* g resp. at 0, 10, 30 and 100 mg/kg bw/d in main groups and 1.6930 and 1.7471 g resp. at 0 and 100 mg/kg bw/d in satellite groups). A slight decrease ovary weight was noted (0.1259, 0.1264, 0.1273 and 0.1073g resp. at 0, 10, 30 and 100 mg/kg bw/d in main groups and 0.1358 and 0.1207 g resp. at 0 and 100 mg/kg bw/d in satellite groups). The relative kidneys weight was also significantly higher (0.6991, 0.6965, 0.7145, 0.7844**g resp. at 0, 10, 30 and 100 mg/kg bw/d in main groups and 0.6572 and 0.7013g resp. at 0 and 100 mg/kg bw/d in satellite groups). Furthermlore, the relative ovary weight was also significantly affected (0.0548, 0.0518, 0.0543, 0.0474*g resp. at 0, 10, 30 and 100 mg/kg bw/d in main groups and 0.0.0525 and 0.0484 g resp. at 0 and 100 mg/kg bw/d in satellite groups).

- *Histopathology findings: incidence and severity :* no treatment-related effect observed

3.12.1.3.3 Range-finding study for the 28-day repeated dose toxicity study (Anonymous 38, 1996)

Study reference:

Anonymous 38, 1996

Detailed study summary and results:

Test type

- Range-finding study
- Observation items : mortality, clinical signs, body weight and necropsy findings

Test substance

- 1-nitropropane
- *Degree of purity:* not specified

Test animals

- *Species/strain/sex:* rat / SD / both sexes
- *Nb. of animals per sex per dose :* 3/sex/dose

- *Age and weight at the study initiation :* 138-225 g in males and 126-181 g in females

Administration/exposure

- *Route of administration:* oral (gavage)
- *Duration and frequency of test/exposure period :* up to 14 D
- *Doses/concentration levels :* 0, 10, 50, 150 and 250 mg/kg bw/d
- *Post exposure observation period :* /
- *Vehicle:* arachis oil

Results and discussion

- *Mortality and time to death :* one male killed in extremis on D 7 at 150 mg/kg bw/d and all animals killed in extremis at the highest dose (2 females on D 4, 1 male on D 6 and the remaining on D 9)
- *Description, severity, time of onset and duration of clinical signs :* no treatment related effects was observed at 0, 10 and 50 mg/kg bw/d. At 150 and 250 mg/kg bw/d, animals exhibited pilo-erection, pallor of the extremities, ataxia, body tremors, loss of righting reflex . At 250 mg/kg bw/d, hunched posture, lethargy, decreased respiratory rate, ptosis, dehydratation, emaciation were also observed.
- *Body weight and body weight changes :* lower bw was observed at the highest dose at D 4 and 8
- *Food/water consumption :* no information available
- *Sensory activity, grip strength and motor activity assessments :* not examined
- *Ophthalmologic findings:* not examined
- *Haematological findings:* not examined
- *Clinical biochemistry findings:* not examined
- *Gross pathology findings:* at the 2 highest doses, necropsy findings were observed : pale kidneys, pale liver (only at 250 mg/kg bw/d), pale adrenals (only at 250 mg/kg bw/d), epithelial sloughing of the non-glandular region of stomach
- *Histopathology findings:* not examined

3.12.2 Human data

3.12.2.1 Page *et al.*, 2001

A case report was published by Page *et al.* in the American Journal of Industrial Medicine ("Peripheral neuropathy in workers exposed to nitromethane", American Journal of Industrial Medicine, 40, 107-113, 2001).

Two workers had large dermal and inhalation exposure to nitromethane during 1 to 2 months. Nitromethane was used as a spray to wipe out excess of glue off headlights. Men were exposed around 55 to 60h/week, in average and were only equipped of aprons and protective glasses. Severe axonal neuropathy was diagnosed after electromyography, nerve conduction studies and medical evaluation. Nitromethane exposure is likely to be the cause of the development of these symptoms, according to the authors, but co-exposure with other chemicals cannot be excluded.

3.12.3 Other data

3.12.3.1 Other data regarding NITROETHANE

3.12.3.1.1 Neurotoxicity study (Kanada *et al.*, 1994)

Study reference:

Kanada *et al.*, 1994. Neurochemical Profile of Effects of 28 Neurotoxic Chemicals on the Central Nervous System in Rats (1) Effects of Oral Administration on Brain Contents of Biogenic Amines and Metabolites, Industrial health, 32, 145-164.

Detailed study summary and results:

Test type

- Disregarded study because origin of the effects are not described (direct/indirect effect due to hypoxia)
- Not GLP-compliant
- Reliability 4 (according to the registration dossier)

Test substance

- Nitroethane
- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* rat / Sprague-Dawley / male and female
- *Nb. of animals per sex per dose:* 4-5

Administration/exposure

- *Route of administration – oral :* gavage
- *Duration and frequency of test/exposure period:* single dose
- *Doses/concentration levels, rationale for dose level selection:* 275 mg/kg bw
- *Control group and treatment:* no treatment

Description of test design:

- *Details on mating procedure:* N/A
- *Post exposure observation period:* 2 h
- Exposure by gavage, then sacrifice by microwave irradiation on the head. Brains were examined.

Results and discussion

- Increased levels of MHPG and 5HIAA in treated groups
- But as it was previously shown that nitroethane administered repeatedly could cause elevated methemoglobinenia, it is complicated to conclude if it is due to a direct effect of nitroethane or indirect via a decrease in oxygen levels in the brain

3.12.3.1.2 Hepatotoxicity study (Dayal R. *et al.*, 1989)

Study reference:

Dayal, R., Gescher, A., Harpur, E.S, Pratt, I., and Chipman, K., 1989. Comparison of the Hepatoxicity in Mice and the Mutagenicity of Three Nitroalkanes. Fundamental and Applied Toxicology, 13, 341-348.

Detailed study summary and results:

Test type

- Not following guideline
- GLP : not specified
- Reliability 2 (according to the registration dossier, but reporting deficiencies (doses not clearly stated for example))

Test substance

- Nitroethane
- *Degree of purity:* unknown

Test animals

- *Species/strain/sex:* BALB/c mice / male and female
- *Nb. of animals per sex per dose :* 3 to 5 (19-25 g)

Administration/exposure

- *Route of administration :* intraperitoneal – injection of the compounds between 9 and 11 AM in a volume of 0.2 mL.
- *Duration and frequency of test/exposure period:* single dose
- *Doses/concentration levels, rationale for dose level selection:* 4.5, 6.7 or 9.0 mmol/kg;
- *Control group and treatment:* control mice were injected with NaCl (0.9% w/v)
- *Vehicle:* physiological saline
- *Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation* : analytical monitoring not specified

Description of test design:

- *Details on mating procedure:* N/A
- *Post exposure observation period:* Mice were sacrificed 24 - 96 hours after dosing

Results and discussion

- No significant increase in SDH, ALT or AST activity noted in mice dosed with nitroethane.
- The livers of mice which had received nitroethane (9 mmol/kg) did not show significant abnormalities

3.13 Aspiration hazard

Hazard class not assessed not assessed in this dossier

4 ENVIRONMENTAL HAZARDS

Not evaluated in this CLH dossier.

5 ABBREVIATIONS

*	P<0.05
**	P<0.01
***	P<0.001
± SD	± Standard deviation
5 HIAA	5-hydroxyindolacetic acid
A/G	Albumin/globulin
Abs	Absolute
Aggreg	Aggregate
ALP	Alkaline phosphatase
ALT	Alanine Transaminase
Alv	Alveolar
Approx.	Approximately
AST	Aspartate Transaminase
ATE	Acute toxicity estimate
Avg	Average
Bronch	Bronchiolar
Bili	Bilirubin
Bronch	Bronchiolar
BUN	Blood urea nitrogen
BW	Body weight
BWG	Body weight gain
Carc.	Carcinogen
Cat.	Category
CE	Cloning efficency
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
Chrom.	Chromosome
CMC	Carboxymethylcellulose
Conc.	Concentraton
CP	Cyclophosphamide
Creat	Creatinine
CT	Clotting time
CWR	Case western reserve university
D or d	Day
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
DS	Dossier submitter
E. Coli	Escherichia coli
epith	Epithelium

Ext	External
F	Female
FBW	Final body weight
G	Gram
GD	Gestational day
GLP	Good laboratory practices
Gp	Group
H	Hour
Hb	Hemoglobin
HCD	Historical control data
HGPRT or HGPRT	Hypoxanthine-guanine phosphoribosyltransferase
HPC	Hepatocyte culture
Htc or Ht	Hematocrit
IC95	Interval confidence of 95 %
Impl.	Implantation
Inc	Incidence
Infla	Inflammation
IM	Intra-muscular
IP	Intra-peritoneal
L.	Left
LC50	Lethal concentration 50%
LC100	Lethal concentration 100 %
LCLo	Lowest lethal concentration
LD	Lactation day
LD0	Lethal dose 0 %
LD50	Lethal dose 50 %
LD100	Lethal dose 100 %
LOAEC	Low observed adverse effect concentration
LOAEL	Low observed adverse effect level
Lymph	Lymphocyte
M	Male
Macro	Macroscopic
Malf.	Malformation
Max.	Maximum
MCV	Mean corpuscular volume
Met. Act.	Metabolic activation
MetHb	Methemoglobin
MHPG	3-Methoxy-4-hydroxyphenylglycol
Min	Minimum
MMAD	Mean mass aerodynamic diameter
MMC	Mitomycine
MN	Micronuclei
MNBC	Micronucleated binucleated cells
Mononucl	Mononuclear
N or Nb	Number
N/A	Not applicable

Nb	number
NC	Negative control
NCE	Normochromatic erythrocyte
ND	Not determined
NE	Not evaluated
NM	Nitromethane
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOEC	No observed effect level
NZW	New Zealand White
O.E.	Olfactory epithelium
Obs	Observation
OCT	Ornithine Carbamyl Transferase
OECD TG	OECD test guideline
Olf	Olfactive
PC	Positive control
PCV	Pack cell volume
PCE	Polychromatic erythrocyte
Plt	Platelet
PND	Post Natal day
Pos	Positive
Ppm	Part per million
Prot	Protein
PT	Prothrombine
R.E.	Respiratory epithelium
RBC	Red blood cell
RCS	Relative cell survival
Rel	Relative
Resp.	respectively
Ret	Reticulocyte
RPE	Relative plating efficiency
S. typh.	Salmonella typhimurium
SCE	Sister chromatide exchange
SD	Sprague-Dawley
SDH	Sorbitol Dehydrogenase
SEM	Standard error of the mean
SHE	Syrian hamster embryo
Skel	Skeletal
St. Dev.	Standard deviation
T3	Triiodothyronine
T4	Tyroxine
TG	Test guideline
Tot.	Total
Tri	Triglyceride
UDS	Unscheduled DNA synthesis
WBC	White blood cell

Wk	week

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