CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1-Nitropropane

EC Number: **203-544-9**

CAS Number: **108-03-2**

Index Number: **609-001-00-6**

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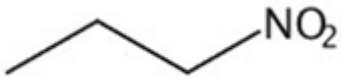
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1-Nitropropane
Other names (usual name, trade name, abbreviation)	1-Nitropropan
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	203-544-9
EC name (if available and appropriate)	1-Nitropropane
CAS number (if available)	108-03-2
Other identity code (if available)	/
Molecular formula	C3H7NO2
Structural formula	
SMILES notation (if available)	/
Molecular weight or molecular weight range	89.09 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	/
Description of the manufacturing process and identity of the source (for UVCB substances only)	/
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 98 % (w/w)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
1-Nitropropane EC n° 203-544-9	≥ 98 - ≤ 100 %	Flam. Liq. 3, H226 Acute Tox. 4*, H302 Acute Tox. 4*, H312 Acute Tox. 4*, H332	Flam. Liq. 3, H226 Acute Tox. 4, H302 Acute Tox. 4, H312 Acute Tox. 4, H332

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
See confidential annex to CLH report	/	/	/	Impurities are not relevant for C&L

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 4: proposed harmonised classification and labelling according to CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	609-001-00-6	1-nitropropane	203-544-9	108-03-2	Flam. Liq. 3 Acute Tox. 4* Acute Tox. 4* Acute Tox. 4*	H226 H302 H312 H332	GHS02 GHS07 Wng	H226 H302 H312 H332			
Dossier submitters proposal	609-001-00-6	1-nitropropane	203-544-9	108-03-2	Retain: Flam. Liq. 3 Remove: Acute Tox. 4* Modify : Acute Tox. 4 Acute Tox. 3 Add : Carc. 1B Repr. 1B STOT RE 2	Retain: H226 H302 Remove: H312 Modify: H331 Add: H350 H360Df H373 (blood, respiratory tract and nervous system)	Retain: GHS02 GHS07 Remove: Wng Modify: Dgr Add: GHS08	Retain: H226 H302 Remove: H312 Modify : H331 Add: H350 H360Df H373 (blood, respiratory tract and nervous system)		Add: ATE (oral) = 506 mg/kg bw ATE (inhalation) = 5.50 mg/L (vapour)	
Resulting Annex VI	609-001-00-6	1-nitropropane	203-544-9	108-03-2	Flam. Liq. 3 Acute Tox. 4	H226 H302	GHS02 GHS07	H226 H302		ATE (oral) = 506 mg/kg bw	

entry if agreed by RAC and COM					Acute Tox. 3 Carc. 1B Repr. 1B STOT RE 2	H331 H350 H360Df H373 (blood, respiratory tract and nervous system)	GHS08 Dgr	H331 H350 H360Df H373 (blood, respiratory tract and nervous system)		ATE (inhalation) = 5.50 mg/L (vapour)	

Table 5: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	**Flam. Liq. 3, H226**	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	**Acute Tox. 4, H302**	Yes
Acute toxicity via dermal route	No classification	Yes
Acute toxicity via inhalation route	**Acute Tox. 3, H331**	Yes
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Data inconclusive	Yes
Carcinogenicity	**Carc. 1B, H350**	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Reproductive toxicity	**Repr. 1B, H360Df**	Yes
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	**STOT RE 2, H373 (blood, respiratory tract and nervous system)**	Yes
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

1-Nitropropane is a chemical substance which is registered under REACH (1907/2006/EC). The substance is listed in annex VI of CLP (609-001-00-6) with the following classification :

Flam. Liq. 3, H226
Acute Tox. 4*, H302
Acute Tox. 4*, H312
Acute Tox. 4*, H332

Several self-classifications are reported in the C&L inventory (consulted on the 24-11-2023): the classification in bold represents the one given in the public REACH registration dossier:

Flam. Liq. 3, H226
Acute Tox. 4, H302
Acute Tox. 4, H312
Acute Tox. 4, H332
Acute Tox. 3, H331

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level:

* The substance warrants classification for the carcinogenicity and reproduction. The substance is not yet self-classified for neither of them.

Justification that action is needed at Community level is required:

* Acute toxicity: change in existing entry due to changes in the criteria: current CLH based on minimum classification (translation table).

* STOT RE: Disagreement by DS with current self-classification not including classification for STOT RE.

5 IDENTIFIED USES

Solvent, consumer use in coatings.

6 DATA SOURCES

Registration dossier (consultation by the DS: September 2023; https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/11705)

C&L inventory: consulted by the DS : 24-11-2023

Full study report

7 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101.3 kPa	Colourless organic liquid with a mild odour	Anonymous 1	Reliability 2 (reliable with restrictions)
Melting/freezing point	-104 °C	Anonymous 2 (1955)	Reliability 2 (reliable with restrictions) Non-guideline, Non-GLP
	-108 °C	Budavari (1989), The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals	Reliability 2 (reliable with restrictions) Data published in peer-reviewed literature
	-108 °C	Lide (2003), CRC Handbook of Chemistry and Physics	Reliability 2 (reliable with restrictions) Data published in peer-reviewed literature
Boiling point	131.2 °C at 1019 hPa	Anonymous 2 (1955)	Reliability 2 (reliable with restrictions)

Property	Value	Reference	Comment (e.g. measured or estimated)
			Non-GLP, non-guideline, data from a collection
	131.1 °C at 1013 hPa	Lide (1995), CRC Handbook of Chemistry and Physics	Reliability 2 (reliable with restrictions) Data published in peer-reviewed literature
	131.6 at 1 atm	Lide (2000), CRC Handbook of Chemistry and Physics	Reliability 2 (reliable with restrictions) Data published in peer-reviewed literature
Relative density	1 g/cm^2 at 25 °C	Anonymous 2 (1955)	Reliability 2 (reliable with restrictions) Non-GLP, non-guideline, data from a collection
	0.99 g/cm^2 at 25 °C	Budavari (1989), The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals	Reliability 2 (reliable with restrictions) Data published in peer-reviewed literature
Vapour pressure	13.64 hPa at 25 °C	Anonymous 2 (1955)	Reliability 2 (reliable with restrictions) Non-GLP, data from a collection
	13 hPa at 25 °C	Dauber and Danner (1989), Physical and Thermodynamic Properties of Pure Chemicals Data Compilation	Reliability 2 (reliable with restrictions) Data published in peer-reviewed literature
	10 hPa at 20 °C	Lide (2003), CRC Handbook of Chemistry and Physics	Reliability 2 (reliable with restrictions) Non-GLP, data were obtained from a standard literature reference on physical properties
Surface tension	67.2 mN/m at 21.6 °C	Anonymous 3 (2010)	Reliability 1 (reliable without restriction) GLP OECD TG 115 (ring method)
Water solubility	1.5 (wt%) at 25 °C	Anonymous 2 (1955)	Reliability 2 (reliable with restrictions) Non-GLP, non-guideline, data from a collection
	15000 mg/L at 25 °C	Lide (2003), CRC Handbook of Chemistry and Physics	Reliability 2 (reliable with restrictions) Data published in peer-reviewed literature
	14000 mg/L	Budavari (1989), The Merck Index – Encyclopedia of Chemicals, Drugs and Biologicals	Reliability 2 (reliable with restrictions) Non- GLP, data were obtained from a standard literature reference on physical properties

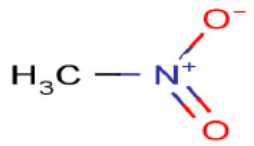
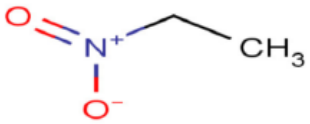
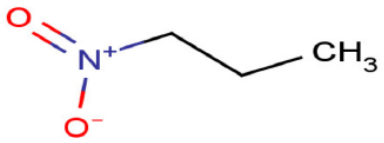
Property	Value	Reference	Comment (e.g. measured or estimated)
Partition coefficient n-octanol/water	Log Kow= 0.79 at 22 °C, pH 7	Anonymous 4 (1994)	Reliability 1 (reliable without restriction) GLP EU A.8 (Partition Coefficient) shake flask method to flask method
	Log Kow= 0.95 at 20 °C, pH 7	Anonymous 5 (2003)	Reliability 2 (reliable with restrictions) Non-GLP, estimation (KOWIN v1.67)
Flash point	36 °C at 101.21 kPa	Anonymous 3 (2010)	Reliability 1 (reliable without restriction) GLP EU A.9 (Flash point)
	36 °C	Fire Protection Guide to Hazardous Materials. 13 ed. Quincy, MA: National Fire Protection Association (2002)	Reliability 2 (reliable with restrictions) Data from handbook or collection of data with peer review Closed cup method
	38 °C at 1 atm	Anonymous 6 (1973)	Reliability 2 (reliable with restrictions) ASTM method D93 (closed cup)
Flammability	/	/	/
Explosive properties	Non-explosive	Anonymous 3 (2010)	Reliability 1 (reliable without restriction) GLP EU A.14 (Explosive properties)
Self-ignition temperature	> 400 °C at 101.21 kPa	Anonymous 3 (2010)	Reliability 1 (reliable without restriction) GLP EU A.15 (Auto-Ignition Temperature (Liquids and Gases))
Oxidising properties	There are no chemical groups that would imply oxidising properties.	/	/
Granulometry	Substance is a liquid	/	/
Stability in organic solvents and identity of relevant degradation products	/	/	/
Dissociation constant	/	/	/
Viscosity	/	/	/

Read-across justification between nitromethane, nitroethane and 1-nitropropane:

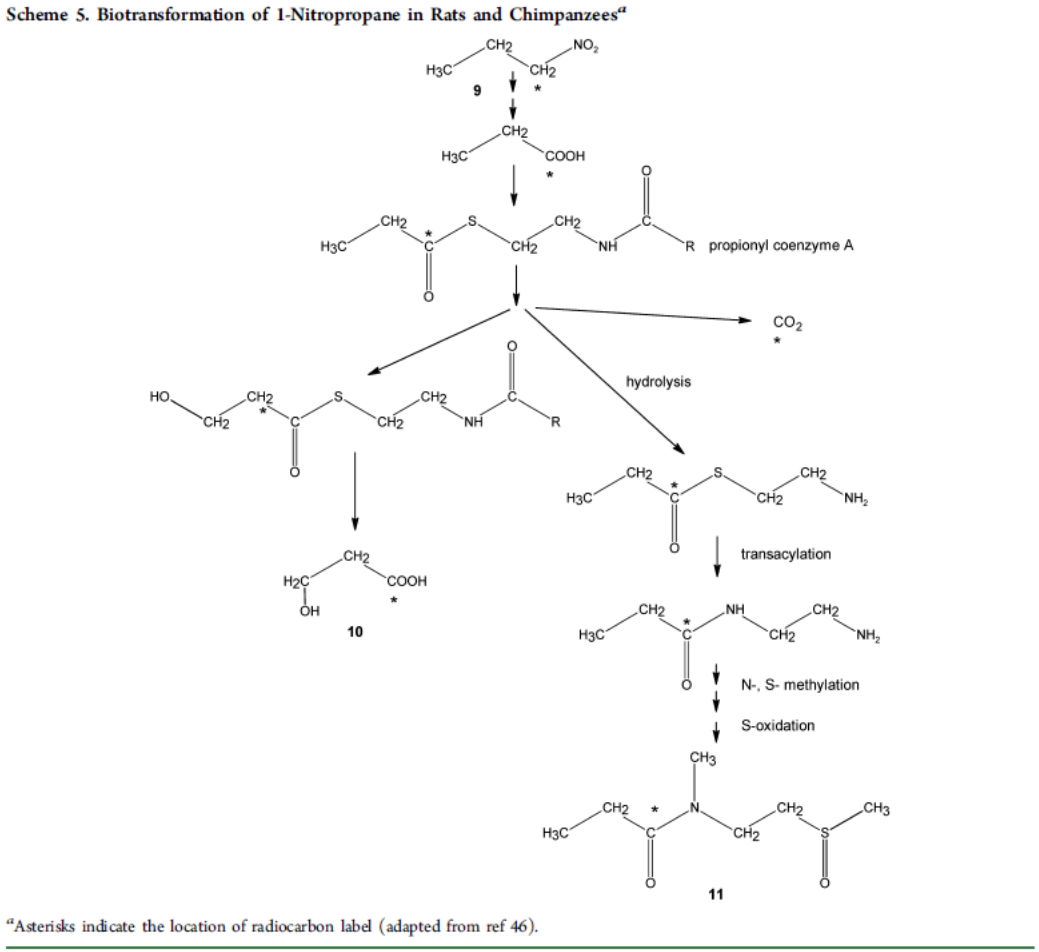
The read-across approach is considered appropriate by the Dossier Submitter as well as the REACH registrants between the members of the short chained nitroparaffins, namely: nitromethane, nitroethane, and 1-nitropropane. These substances share similar structure and properties including toxicological properties as shown by the toxicological data when available for all substances (see e.g. acute oral and inhalation toxicity and STOT RE). This category approach has also been accepted by the OECD SIAM October 2010 "*The short chain nitroparaffins category consists of three structurally related nitroalkanes; nitromethane, nitroethane and 1- nitropropane. These chemicals are considered a category because of the similarities in structure, and in chemical and toxicological behaviour. The category members are expected to be absorbed, metabolized, and excreted in a similar fashion, resulting in the release of their respective aldehydes and nitrite.*"

All three nitroalkanes are straight alkyl chain with similar molecular weights and only one single common functional group (Table 7). The only structural difference between nitromethane and nitroethane is a one carbon addition to the alkyl group. Further analogues differ in the length of the alkyl group so that the following sequence is obtained: from 0 carbon atoms (NM) through 1 (NE) to 2 (1-NP). There are no other functional groups present in these molecules. They have a common breakdown pathway to nitrite and corresponding aldehyde (Smith & Anderson, 2013 - Figure 1), which are also expected to have similar toxicological properties based on structural similarity. The category members are expected to be absorbed, metabolized, and excreted in a similar fashion, resulting in the release of their respective aldehydes and nitrite.

Table 7: Identification and structures of structurally similar substances

Substances:	N° CAS:	Molecular weight:
Nitromethane	75-52-5	61.04 g/mol
Nitroethane	79-24-3	75.07 g/mol
1-Nitropropane	108-03-2	89.09 g/mol
Structures:		
Nitromethane	**Nitroethane**	**1-Nitropropane**
		
Physical state		
Liquid	Liquid	Liquid

Melting point (°C)		
-28.4 °C	-89.5 °C	-104 °C
Boiling point (°C)		
101.2 °C at 1013 hPa	114 °C at 1013 hPa	131.1 °C at 1013 hPa
Density		
1.1322 g/cm³ at 25 °C	1.0448 g/cm³ at 25 °C	0.9934 g/cm³ at 25 °C
Vapour pressure (hPa)		
37.1 hPa at 20 °C	27.7 hPa at 25 °C (estimated)	13 hPa at 25 °C (estimated)
Water solubility (g/L at 20 °C)		
111000 mg/L at 20 °C	45000 mg/L at 20 °C	15000 mg/L at 25 °C
Partition coefficient n-octanol/water (log value)		
-0.33	0.18	0.79
Henry's law constant		
2.1 Pa*m³/mol (estimated)	4.7 Pa*m³/mol	2.1 Pa*m³/mol (estimated)



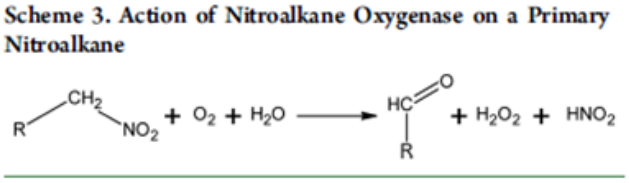


Figure 1: Biotransformation of 1-nitropropane as proposed by Smith & Anderson (2013)

As described in Smith & Anderson paper (2013) on nitroalkanes metabolism, denitrification of these nitrocompounds may lead to the release of a sufficient quantity of nitrite to induce transient methemoglobinemia. Moreover, acute and chronic exposure to nitromethane, nitroethane or 1-nitropropane (also called nitroparaffins) have led to liver and kidney damage, central nervous system depression, eyes and respiratory system irritation.

Also, as reported in the paper, nitromethane and another nitroparaffin (2-nitropropane) can reasonably be expected to be human carcinogens.

About ADME, these nitroparaffins are not expected to be caustic and induce local contact toxicity. Toxicity usually comes from absorption and metabolism of the parent compound into nitrite and an aldehyde.

Around 17 % of parent radiolabeled 1-nitropropane (number 9 in Figure 1) was excreted with 15 % in the urine and 2 % in feces. It was concluded that biliary elimination of parent compound or its metabolites was a minor route of elimination while the major route was identified as the respiratory tract with a recovery of 75 % of the radioactivity. This was similar in rats and in chimpanzees. Furthermore, in rats, 14.2 % of the expired radiolabeled fraction was 1-nitropropane and it represented around 10 % of the total radioactive dosed compound.

Two major metabolites were identified as numbers 10 and 11 in Figure 1, respectively 3-hydroxypropionic acid and N-methyl-N-2-(methylsulfinyl)ethyl propionic acid amide (NMPA). Three other metabolites were detected but not identified and propionaldehyde was not detected. The first metabolic step in animals was determined as denitrification, probably via cytochrome P450 reactions.

Nitrate/nitrite toxicity has been extensively reviewed by international organizations (e.g. WHO for the purpose of development of WHO Guidelines for drinking water quality, Health Canada, WHO for the purpose of food additives assessment and nitrosamine formation, US ATSDR). There are indications for common mode of action-mediated effects for a number of substances containing nitrate (including dinitrite glycerol) regarding:

- Spermatotoxic and fertility related effects involving NO redox cycle
- Thyroid effects due to displacement of iodine
- Carcinogenic effects

In these three nitroalkanes, differences in toxicity can arise from the metabolic byproducts of aldehydes which are also close analogues as such, however, no common compounds include formaldehyde, acetaldehyde, and propanaldehyde and no effects are seen that can be further attributed to these aldehydes. Nevertheless, at high doses it can be expected that the presence of metabolic products like the aldehydes would contribute to some extent to the toxicity. The three aldehydes have a common mode of action with cytotoxicity and creation of Reactive Oxygen Species.

The Registrant submitted in addition to the CSR a Read-Across justification document in line with the principles described in ECHA guidance and practical guides which is considered as sufficiently detailed and is supported by the DS as the submitted information is adequate to characterize the read across plausibility of nitroalkanes. Indeed, the read-across is further supported by experimental ADME data, physico-chemical properties and systemic toxicity findings. The *Read-across justification document* was made available to the DS by the registrant and is attached within the confidential annex I to this CLH dossier.

For acute oral and inhalation toxicity, there is conclusive data on each of the category members, and thus classification proposals for acute toxicity for each of the category members is based on the data on the substance itself.

The classification proposal for carcinogenicity of nitroethane and nitropropane is fully based on read-across from nitromethane because the available studies on nitroethane and nitropropane are uninformative due to too low dosing and too low animal number. Thus, the key studies for the assessment of

carcinogenicity are the 2-year studies in mice and rats on nitromethane for all category members and Carc. 1B; H350 are proposed for nitromethane, nitroethane and nitropropane in individual dossiers.

The classification proposal for sexual function and fertility of nitromethane, nitropropane and nitroethane is based on the overall WoE from all category members. There is no EOGRTS or 2-generation study on any of the category member, and thus only limited aspects of potential effects on sexual function and fertility have been investigated in the available data set. However, spermatotoxic effects were reported on nitromethane (90-day NTP studies in rats and mice) and nitroethane (90-day NTP study in rats) and these findings are supported by nitrate/nitrite-mediated spermatotoxic and fertility related effects involving NO redox cycle. As indicated above, nitrite is the common metabolite for nitromethane, nitroethane, and 1-nitropropane. In addition, the OECD TG 422 on 1-nitropropane showed that 2 females at the mid- and high dose groups failed to become pregnant. Overall, these data are considered to support Repr. 2; H361f for nitromethane, nitroethane and 1-nitropropane and these classifications are proposed in individual dossiers.

The classification proposal for developmental toxicity of nitroethane and nitropropane is fully based on read-across from nitromethane OECD TG 414 study in rats, because there is no prenatal developmental toxicity study available on nitroethane and 1-nitropropane. Overall, the available data on nitromethane is considered to support Repr. 1B; H360D for nitromethane, nitroethane and nitropropane and these classifications are proposed in individual dossiers.

Studies investigating effects on respiratory tract, blood and nervous system are available on each of the category member and they show consistent effects at comparable doses (within GV range for category 1 and 2). Also read-across between category members is considered justified and the effects on respiratory tract, blood and nervous system occur within the GV range for classification in category 1 and 2 also when the effective dose for a target substance is calculated based on its molecular weight. All in all, classification as STOT RE 2, H372 (respiratory tract, blood and nervous system) is considered warranted for nitromethane, nitroethane and nitropropane and these classifications are proposed in individual dossiers.

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this CLH dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Skin absorption and metabolism Monkey (macaca fascicularis) females 1-nitropropane (purity: 98 %) Vehicle: grain alcohol	Excretion: primarily excreted in the urine.	Most of the test material evaporated from the test site although it was covered airtight with a patch during the first twelve hours.	Anonymous 7, 1990

Method	Results	Remarks	Reference
Duration of exposure: 12 h Dose: 15.28 mg No guideline followed GLP-compliant Reliability 2 (according to the registration dossier)		Animals acted very differently in their excretion and absorption behaviour.	
1-nitropropane concentration in liver tissue (Inhalation exposure) 4 SD male rats 1-nitropropane Vehicle: alcohol solution Duration of exposure: 6 h No guideline followed Not GLP	After 6 h of exposure at 1200 ppm, 50 ppm of 1-nitropropane were accumulated in the liver. 1-nitropropane level increased at 2 h, then began to decrease. The study mentions that 1-nitropropane would disappear after 8-10 h.	The results cannot be verified as the document made available to the DS is poorly reported.	Anonymous 8, 1977
In vitro study using microsomal drug monooxygenase system Rat (SD) Male (number: unknown) 2-nitropropane Vehicle: ethanol Duration of exposure: test ran for 8 min Doses: 0.05 M No guideline followed GLP compliance unspecified Reliability 2 (according to the registration dossier)	Rat liver microsomes in the presence of NADPH and dioxygen catalyse the oxidase denitrification of 2-nitropropane to acetone and nitrite.	Full study not available. Poorly reported data in the registration dossier. No information on the relevance of the read-across from 2-NP to 1-NP. Study not assessed by the DS.	Ulrich V. *et al.*, 1978

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

In a GLP non-guideline dermal absorption study (Anonymous 7, 1990), a single dermal dose (300 µL ether/ethanol solution containing 5 % 14^C 1-nitropropane) 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 the 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. Seventy-two 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 of damage or irritation.

The major route of excretion was urine (approx. 94 %). After 36 hours, no detectable amount of 1-nitropropane was observed in blood .

The very high loss of test material was probably due to the high volatility of the test substance. As animals acted very differently in their excretion and absorption behaviour, no average was determined in the study.

A study performed in male SD rats to test 1-nitropropane concentration in liver tissue after air inhalation (Anonymous 8, 1977) revealed that after 6 hours of exposure to 1200 ppm of 1-nitropropane, liver accumulated 50 ppm of the test substance. Moreover, 1-nitropropane increased at 2 hours, then began to decrease. The study mentions that 1-nitropropane would disappear after 8-10 h.

The results cannot be verified as the data made available to the DS is poorly reported.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity – oral route

Table 9: Summary table of animal studies on acute oral toxicity

Method, guideline	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity study Gavage No guideline followed GLP-compliant Reliability 1 (according to the registration dossier, however only summary data available to the DS)	Rat/SD/both sexes 10/sex/dose	**1-nitropropane** Purity: 96.12 % Vehicle: 1 % CMC	Doses: 0, 290, 360, 450 and 570 mg/kg bw Single exposure Post-exposure period: 14 d	**LD50 (M/F): 506 mg/kg bw** **LD50 (M): 528 mg/kg bw** **LD50 (F): 484 mg/kg bw**	Anonymous 9, 1981
Acute oral toxicity study No guideline followed	Rat No more information	**1-nitropropane** Purity:	Unspecified	**LD50: 280 – 420 mg/kg bw** (no additional results were reported)	Anonymous 10, 1956

Method, guideline	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Not GLP Full study not available Reliability 4 (according to the registration dossier)	available	unspecified			
Acute oral toxicity study No guideline Not GLP Full study not available Reliability 4 (according to the registration dossier)	Rat No more information available	**1-nitropropane** Purity unspecified	Unspecified	**LD50: 380 – 530 mg/kg bw** Significant liver damage Lung haemorrhage	Anonymous 11, 1960
Acute oral toxicity study Gavage No guideline Not GLP Article was available to the DS; however, this article is mostly not readable due to the bad quality of the PDF file Reliability 4 (according to the registration dossier)	Rabbit No more information available	**1-nitropropane** Purity: unspecified	Unspecified	**LD50: 250 – 500 mg/kg bw** Clinical signs observed 20 – 40 min after exposure (weakness, incoordination, ataxia, bradypnea)	Machle W. *et al*., 1940

No human data or other data available.

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In an acute oral toxicity study (Anonymous 9, 1981), groups of 10 male and 10 female rats were given by gavage 1-nitropropane at a concentration of either 0, 290, 360, 450 or 570 mg/kg bw. Animals were observed during 14 days.

Mortality was observed at the 2 highest dose levels. 3 males and 3 females exposed to 450 mg/kg bw and 6 males and 9 females exposed to 570 mg/kg bw were found dead. The deaths occurred within 36 h after treatment. Necropsy of these animals revealed distended and haemorrhagic intestines. Furthermore, necropsy of surviving animals showed lung infection in 3, 1, 5 and 1 animals at 290, 360, 450 and 590 mg/kg bw, respectively.

In this study, the calculated LD50 was 506 mg/kg bw for both sexes. The LD50 for males was of 528 mg/kg bw while the LD50 for females was of 484 mg/kg bw.

Other acute oral toxicity studies with minimal description of methods and results reported LD50 values of 280 – 420 mg/kg bw in rats (Anonymous 10, 1956), 380 – 530 mg/kg bw (Anonymous 11, 1960) and 250 – 500 mg/kg bw in rabbits (Machle W. *et al.*, 1940).

The test substance was previously classified as Acute Tox. 4*, H312.

10.1.2 Comparison with the CLP criteria

CLP criteria	Results of available studies
Acute toxicity category 4: oral LD50: > 300 but ≤ 2000 mg/kg bw	LD50 of all the available studies were within the range of the Category 4 Key study (Anonymous 9, 1981): LD50 (both sexes) of 506 mg/kg bw

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available results, a classification as **Acute Tox. 4; H302 (Harmful if swallowed)** is proposed as well as an **ATE of 500 mg/kg bw**, based on the CLP regulation.

10.2 Acute toxicity - dermal route

Table 10: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
Acute dermal toxicity	Rabbit (strain not	**1-**	Dose: 2000 mg/kg bw	**LD50 > 2000**	Anonymous

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference
study Occlusive No guideline followed No information about GLP compliance Reliability 1 (according to the registration dossier)	reported) 4 males and 5 females	**nitropropane** Purity: 96.12 %	Duration of exposure: 24 h	**mg/kg bw** No signs of irritation	12, 1981
Acute dermal toxicity study No guideline followed Not GLP Full study not available Reliability 4 (according to the registration dossier)	10 Rabbits (strain and sex unspecified)	**1-nitropropane** Purity: unknown	Doses: not reported Duration of exposure: not reported	**LD50 > 200 mg/kg bw**	Anonymous 10, 1956
Acute dermal toxicity study Open No guideline followed Not GLP Article was made available to the dossier submitter; however, this article is mostly not readable due to the bad quality of the PDF file Reliability 4 (according to the registration	Rabbit (strain and sex not reported)	**1-nitropropane** Purity: unknown	Doses: not reported Duration of exposure: 5 treatments in 5 d	Mortality: not reported Clinical signs and necropsy: no effects observed	Machle W. *et al*., 1940

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference
dossier)					

No human data or other data available.

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In an acute dermal toxicity study (Anonymous 12, 1981), 4 male and 5 female rabbits were exposed to 2000 mg/kg bw of 1-nitropropane during 24 hours. All animals survived and did not exhibit signs of irritation. The LD50 was of > 2000 mg/kg bw.

Two other dermal toxicity studies with minimal description of methods and results reported a LD50 higher than 200 mg/kg bw (Anonymous 10, 1956) and no mortality (Machle *et al.*, 1940) (no more information available).

The test substance was previously classified as Acute Tox. 4*, H312.

The test substance is known to be highly volatile. This aspect is not discussed in briefly reported Anonymous 12 (1981) study but it is stated that impervious material was used to cover the testing site to prevent any test substance loss.

10.2.2 Comparison with the CLP criteria

CLP criteria	Results of available studies
Acute toxicity category 4: dermal LD50: > 1000 but ≤ 2000 mg/kg bw	No mortality observed in the available studies (LD50 > 2000 mg/kg bw)

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the available results, a classification as Acute toxicity via dermal route may not be warranted.

10.3 Acute toxicity - inhalation route

Table 11: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity study Vapour No guideline followed Not-GLP Reliability 2 (according to the registration dossier, however only summary data available to the DS, no individual data)	Rat (Wistar) 6 females/dose	**1-nitropropane** Purity: 96.12 % Vapour	Doses: 8.60, 11.02 and 14.40 mg/L Duration of exposure: 1 h	**LC50: 11.02 mg/L** Mortality: 0, 3 and 5 females resp. at 8.60, 11.02 and 14.40 mg/L (1 h after exposure)	Anonymous 10, 1956
Acute inhalation toxicity study No guideline followed Not GLP Reliability 2 (according to the registration dossier, however only summary data available to the DS, no individual data)	Rat (Wistar) 8-10 animals/dose	**1-nitropropane** Purity: not reported	Doses: 80, 400, 800, 1100, 2500 and 13000 ppm approx. equivalent to 0.29, 1.46, 2.91, 4.01, 9.11 and 47.37 mg/L, resp. Nb of exposure: 8, 8, 3, 1, 1 and 1 resp. at 80, 400, 800, 1100, 2500 and 13000 ppm Duration of exposure: 8, 8, 6, 11, 8 and 5 h resp. at 80, 400, 800, 1100, 2500 and 13000 ppm	**LC100: 2500 ppm (1 exposure of 8 h) and 1100 ppm (1 exposure of 11 h)**	Dequidt J. *et al.,* 1973
Acute inhalation toxicity study No guideline followed Not GLP Article was made available to the DS; however, this article is mostly not readable due to the bad quality of the PDF file Reliability 4 (according to the registration dossier)	Monkey, rabbit and guinea pig (strain unknown)	**1-nitropropane** Purity: not reported	Doses: max conc. of 1 % Duration of exposure: 3 h	A mass concentration of 1.5 was sufficient to kill all animals and a mass concentration of 1 cause the death of 1 animal	Machle W. *et al*., 1940

No human data or other data available

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In an acute inhalation toxicity study (Anonymous 10, 1956), groups of 6 female rats were exposed to 8.60, 11.02 or 14.40 mg/L of 1-nitropropane during 1 hour. 0, 3 and 5 females died 1 hour after exposure respectively at 8.60, 11.02 and 14.40 mg/L. The LC50 was of 11.02 mg/L.

In another acute inhalation toxicity study (Dequidt J. *et al.*, 1973), groups of 8 to 10 rats were exposed to 80 (8 exposures), 400 (8 exposures), 800 (3 exposures), 1100 (1 exposure), 2500 (1 exposure) or 13000 (1 exposure) ppm of 1-nitropropane. 80, 400, 800, 1100, 2500 and 13000 ppm were approximatively equivalent to 0.29, 1.46, 2.91, 4.01, 9.11 and 47.37 mg/L, respectively. The total duration of exposure was of 8, 8, 6, 11, 8 and 5 h, respectively at 80, 400, 800, 1100, 2500 and 13000 ppm. The LC100 were of 2500 ppm (1 exposure of 8 h) and 1100 ppm (1 exposure of 11 h).

One other study with minimal description of method and results was disregarded (Machle *et al.*, 1940).

The test substance was previously classified as Acute Tox. 4*, H332.

10.3.2 Comparison with the CLP criteria

CLP criteria (vapours)	Results of available studies
Acute toxicity category 3: inhalation LC50: > 2.0 but ≤ 10 mg/L after a 4-hour exposure	LC50 of 11.02 mg/L (Anonymous 10, 1956) for a 1-hour exposure.
Acute toxicity category 4: inhalation LC50: > 10 but ≤ 20 mg/L after a 4-hour exposure	According to the CLP regulation (see Notes c) related to Table 3.1.1.), the derived ATE for a 1-hour exposure can be converted to obtain a 4-hour exposure ATE by dividing by a factor 2. The obtained ATE is 5.51 mg/L.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the available results, a classification as **Acute Tox. 3; H331 (Toxic if inhaled)** is proposed as well as an **ATE of 5.50 mg/L** (vapours), based on the CLP regulation.

10.4 Skin corrosion/irritation

Hazard class not evaluated in this CLH dossier

10.5 Serious eye damage/eye irritation

Hazard class not evaluated in this CLH dossier

10.6 Respiratory sensitisation

Hazard class not evaluated in this CLH dossier

10.7 Skin sensitisation

Hazard class not evaluated in this CLH dossier

10.8 Germ cell mutagenicity

Table 12: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
1-NITROPROPANE				
***In vitro* gene mutation test in bacteria** With and without met. act. OECD TG 471 GLP Protocol adapted to volatile compound Reliability 1 (according to the registration dossier)	**1-nitropropane** Purity: 99 % Vehicle: DMSO	*S. typh.* TA98, TA100, TA1535 and TA1537 and *E. Coli* WP2uvrA- Test conc.: 20, 150, 500, 1500 and 5000 µg/plate	Cytotoxicity: no Genotoxicity: **negative**	Anonymous 31, 1996

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
***In vitro* chromosome aberration study in mammalian cells** With and without met. act. No guideline followed GLP Reliability 2 (according to the registration dossier)	**1-nitropropane** Purity: > 99 % Vehicle: DMSO	Chinese Hamster lung (CHL) cells Test conc.: 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	Cytotoxicity: yes Genotoxicity: **negative**	Anonymous 32, 1994
***In vitro* DNA damage and/or repair study** OECD TG 482 Not GLP Reliability 2 (according to the registration dossier, however not enough information available to the DS, no access to raw data)	1-**nitropropane** Purity: 97.4 % Impurity: 2-nitropropane (2.3 %)	Primary hepatocytes from male and female Wistar rats Test concentrations: 0.1-10 mM	Cytotoxicity: no information available Genotoxicity: **negative**	Andrae *et al.*, 1988
***In vitro* gene mutation test in mammalian cells** OECD TG 476 Not GLP Reliability 2 (according to the registration dossier, however only summary available, not enough information to confirm the validity of the study)	**1-nitropropane** Purity: 97.4 % Impurity: 2-nitropropane (2.3 %)	Chinese hamster lung cells (V79) Test conc.: 0, 0.3, 1, 3, 6 and 10 mM	Cytotoxicity: yes Genotoxicity: **positive**	Roscher *et al.*, 1990
***In vitro* micronucleus test in mammalian cells** OECD TG 487 Not GLP Reliability 2 (according to the registration dossier, however only summary available, not enough information to confirm the	**1-nitropropane** Purity: 97.4 % Impurity: 2-nitropropane (2.3 %)	Chinese hamster lung cells (V79) Test conc.: 0, 0.3, 1, 3, 6 and 10 mM	Cytotoxicity: yes Genotoxicity: **positive**	Roscher *et al.*, 1990

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
validity of the study)				
***In vitro* DNA damage and/or repair study** OECD TG 482 Not GLP Reliability 2 (according to the registration dossier, however only summary available, not enough information to confirm the validity of the study)	**1-nitropropane** Purity: 97.4 % Impurity: 2-nitropropane (2.3 %)	Cell lines of extrahepatic origin, derived from rat (embryonic fibroblasts and carcinoma Walker rat), mouse (embryonic fibroblasts), hamster (fibroblasts lung and fibroblasts ovary) and man (embryonic fibroblasts lung, adenocarcinoma lung, adenocarcinoma lung and epiderm. carcinoma larynx) Test concentrations: 0.1 – 10 mM	Cytotoxicity: unspecified Genotoxicity: **negative**	Andrae U. *et al.*, 1988
***In vitro* gene mutation test in bacteria** With and without met. act. OECD TG 471 GLP Reliability 1 (according to the registration dossier)	**1-nitropropane** Purity: ~99 % Vehicle: DMSO	*S. typh* TA98, TA100, TA1535 and TA1537 and *E. Coli* WP2uvrA- Test conc.: 0, 8, 40, 200, 1000 and 5000 µg/plate (first experiment) and 0, 312.5, 625, 1250, 2500 and 5000 µg/plate	Cytotoxicity: no Genotoxicity: **negative**	Anonymous 33, 1994
***In vitro* gene mutation test in bacteria** With and without met. act. OECD TG 471 Not GLP Reliability 1 (according to the registration dossier, however not enough information to confirme the validity of the study)	**1-nitropropane** Purity: 97 % Vehicle: DMSO	*S. typh.* TA98, TA100, TA1535 and TA1537 Conc.: 0, 100, 333, 1000, 3333 and 10000 µg/plate	Cytotoxicity: no Genotoxicity: **negative**	Haworth S. *et al.*, 1983
NITROMETHANE				
***In vitro* gene mutation test in bacteria** OECD TG 471 Deviation: 4 instead of 5 strains Non-GLP	**Nitromethane** Purity: > 99 %	Pre-incubation test Strain: 4 *S. typh*. strains (TA98, TA100, TA1535 and TA1537) Test conc.: 100, 333.3, 1000, 3333.3 and	No significant increase in the frequency of revertant colonies up to 10 mg/plate, +- S9 Only in TA100, cytotoxicity was observed at the highest concentration tested.	Mortelmans *et al.*, 1986

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Reliability 2 (according to the registration dossier)		10000 µg/plate. +- S9 Vehicle: DMSO	**Negative**	
***In vitro* gene mutation test in bacteria** Prior to OECD TG 471 GLP Reliability 2 (according to the registration dossier, however the study was not made available to the DS. Results should be interpreted with caution)	**Nitromethane** No data on purity	Strain: 5 *S. typh.* strains (TA98, TA100, TA1535, TA1537 and TA1538) Test conc.: 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. +- S9	No significant increase in the frequency of revertant colonies at 23732 ppm, +- S9 **Negative**	Anonymous 27, 1980
***In vitro* chromosome aberration study in mammalian cells** CHO cells OECD TG 473 Non-GLP Reliability 2 (according to the registration dossier)	**Nitromethane** Purity unknown	Cell type: CHO cells Test conc.: 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 +- S9 Vehicle: distilled water	Negative +- S9 at concentrations as high as the limit concentration of 4980 µg/mL **Negative**	NTP, 1997

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
***In vitro* SCE assay in mammalian cells** CHO cells OECD TG 479 non-GLP Reliability 2 (according to the registration dossier)	**Nitromethane** Purity unknown	Cell type: CHO cells Test concentrations: No cytotoxicity was observed at limit concentration ➢ 26-hour treatment without S9: 497, 1655 and 4965 µg/mL then a 2-hour incubation without nitromethane ➢ 2-hour treatment with S9 then incubation was prolonged by 26 h: 497, 1655 and 4965 µg/mL +- S9 Vehicle: distilled water	No induction of SCE in CHO cells +-S9 at concentrations as high as the limit concentration of 4965 µg/mL **Negative**	NTP, 1997
***In vitro* gene mutation test in bacteria** Prior to OECD TG 471 Non-GLP Reliability 2 (according to the registration dossier, however only short data available to the DS)	**Nitromethane** Purity unknown	Strains: 3 *S. typh.* strains (TA1535, TA1537 and TA1538) and 1 *Saccharomyces cerevisiae* (D4)	Disregarded study due to poor data reporting + test material not soluble under the treatment conditions	Anonymous 28, 1975
***In vitro* gene mutation test in bacteria** OECD TG 471 Deviation: only 3 strains tested without met. act. Non-GLP reliability 2 (according to the registration dossier, however reporting deficiences)	**Nitromethane** Purity unknown	Strain: 3 *S.* typh. strains (TA98, TA100 and TA102) Test concentrations: Not specified but up to 200 µmol/plate. Only without S9 Vehicle: not specified	**Negative** without S9	Dayal *et al.*, 1989
***In vitro* cell transformation study in mammalian cells**	**Nitromethane**	cells type: Syrian hamster embryo (SHE)	A dose-dependent significant increase in the morphological transformation frequency seen at	Kerckaert *et al.*, 1996

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
EU Method B.21 Non-GLP Reliability 2 (according to the registration dossier)	Purity unknown	Test conc.: 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	the two highest concentrations tested. **Positive**	
***In vitro* micronucleus test in SHE cells** Non-GLP Reliability 2 (according to the registration dossier)	**Nitromethane** Purity unknown	Cells type: SHE cells Met. act.: not used Test concentrations: - *With DMSO:* 0, 5.0, 5.5 and 6.0 µg/ml - *With Media:* 0, 3500, 4000, 5000 (µg/ml) Vehicle: DMSO or media	Nitromethane did not induce an increased frequency of micronuclei in SHE cells. **Negative**	Gibson *et al.*, 1997
		Results of an additional *in vitro* supporting 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) is considered to be limited and results were negative, the study was not included in this report.		
NITROETHANE				
***In vitro* gene mutation test in bacteria** OECD TG 471 Non-GLP Reliability 2 (according to the registration	**Nitroethane**	*S. typh.* Deviations: only 4 out of 5 strains used (TA98, TA100, TA1535, TA1537) Test conc.: 100, 333.3, 1000, 3333.3 and	Cytotoxicity observed in all 4 strains at 10000 µg/plate Precipitation was observed in the highest concentration tested in most experiments in all the strains	Mortelmans *et al.*, 1986

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
dossier)		10000 µg/plate ± S9 Vehicle: DMSO No negative control Positive controls: -S9: 4-nitro-o-phenylenediamine for TA98, sodium azide for TA100 and TA1535 and 9-aminoacridine for TA1537 +S9: 2-aminoanthracene	Positive control: induced a clear increase in the number of revertants 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), ± S9 **Negative**	
***In vitro* gene mutation test in bacteria** Prior to OECD TG 471 GLP compliant Reliability 2 (according to the registration dossier, however DS not access to raw data)	**Nitroethane**	*S. typh* 5 strains (TA98, TA100, TA1535, TA1537 and TA1538) Conc.: 55450 ppm (vapours) and at least 27725 ppm No vehicle Negative control: unspecified Positive control: -S9: 2-nitrofluorene for TA98 and TA1538; N-methyl-N'-nitro-N-nitrosoguanidine for TA100 and TA1535 and quinacrine mustard-2HCl for TA1537 +S9: 2-acetylaminofluorene for TA98 and TA1538; 2-anthramine for TA100 and TA1535 and 8-aminoquinoline for TA1537	Cytotoxicity was observed at 55450 ppm in TA1535 and TA1537. Therefore, a concentration of 27725 ppm was tested. No significant increase in the frequency of revertant colonies **Negative**	Anonymous 29, 1980
***In vitro* gene mutation study in mammalian cells**	**Nitroethane**	Cells type: CHO Target gene: HGPRT	No cytotoxicity observed at the highest concentration tested	Anonymous 30, 2012

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
OECD TG 476 GLP-compliant Reliability 1 (according to the registration dossier)		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	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. **Negative**	
I*n vitro* gene mutation study in bacteria OECD TG 471 Non-GLP Reliability 2 (according to the registration dossier, however reporting deficiencies)	**Nitroethane**	*S. typh.* Only 3 strains tested (TA98, TA100 and TA102) Conc.: 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) Without met. act.	Negative without met. act. No cytotoxicity observed at any concentration. **Negative**	Dayal *et al.*, 1989

Table 13: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
1-NITROPROPANE				
***In vivo* micronucleus test** No guideline followed Not GLP Reliability 2 (according to the registration dossier, however DS not access to raw data)	**1-nitropropane** Purity: unspecified	Male SD rats (4-8/groups) Gavage Single dose Bone marrow: 24 h: 100, 200, 300 and 400 mg/kg; 48 h: 100, 200 and 300 mg/kg	Genotoxicity: **negative** in the bone marrow, however positive in liver Toxicity: yes, lethality at 500 mg/kg bw	George *et al.*, 1989

Method, guideline	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		Liver: 72 h: 300 mg/kg (lethality observed at 500 mg/kg)		
***In vivo* mammalian cell study : DNA damage and/or repair** No guideline followed Not GLP Reliability 2 (according to the registration dossier, however DS not access to raw data)	**1-nitropropane** Purity: 97.4 % Vehicle: olive oil	Wistar rats 9 controls and 2/sex after 1 h and 17 h IP, single injection Conc.: 20, 40, 60 and 80 mg/kg	Genotoxicity: **negative**	Andrae *et al.*, 1988
***In vivo* mammalian somatic cell study: cytogenicity/erythrocyte micronucleus** No guideline followed GLP compliance unspecified Reliability 2 (according to the registration dossier, however poor quality of the PDF file, difficult to analyse the data)	**1-nitropropane** Purity: unspecified	Mouse (5/sex/group) IP, single dose Conc.: no information available	Genotoxicity: **negative**	Kliesch and Adler, 1987
NITROMETHANE				
***In vivo* micronucleus test in NCEs of B6C3F1 mice** OECD TG 474 Non-GLP 10 males + 10 females Inhalation Reliability 2 (according to the registration dossier)	**Nitromethane** Purity unknown	Treatment: ➢ 6 h/d ➢ 5 d/w for 13 weeks Test conc.: 94, 188, 375, 750 and 1500 ppm (= limit dose) Vehicle: not specified	No increase in the frequency 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. **Negative**	NTP, 1997
		Due to very poor quality of the copy, the study will not be presented in the		Gocke *et al.*, 1981

Method, guideline	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		CLH report and will not be assessed.		
NITROETHANE				
***In vivo* micronucleus test** Prior to OECD TG 474 Prior to GLP CD-1 mice (Charles River) 14/sex/ in control groups 8/sex/dose Reliability 2 (according to the registration dossier, however study not available to the DS)	**Nitroethane**	Oral (gavage) 2x/day Doses: 0.25, 0.5 or 1.00 mL/kg bw/d (highest dose = half the oral LD50 value) Sacrifice 6 h after the last dose Vehicle: unknown Concurrent control: tap water Positive control: methylmethanesulfonate (90 mg/kg bw/d, IP route)	No significant increase in the frequency of micronucleated polychromatic erythrocytes, at doses up to 1 mL/kg bw/d, in either sex. **Negative**	Hite and Skeggs., 1979

No human data available.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagencity

***In vitro* data on 1-Nitropropane**

An *in vitro* gene mutation test in bacteria (Anonymous 31, 1996) was performed using *S. Typh.* (TA98, TA100, TA1535 and TA1537) and *E. Coli* WP2uvrA- with and without metabolic activation. The protocol was adapted to volatile compounds.

In all strains, the positive control compounds induced a clear increase in the number of revertants both in absence and presence of S9 metabolic fraction. 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 (see Table 14).

Table 14: Mean number of revertant colonies

Strain	Dose (µg/plate)	Mean nb of revertants/plate			
		Without met. act.		With met. act.	
		Trial 1	Trial 2	Trial 1	Trial 2
TA 100	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
TA 1535	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
TA 98	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
TA 1537	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

Considering that the test has been performed according to OECD TG 471 and that special adaptations for analyzing volatile compounds were made, it can be concluded that the compound is not-mutagenic under the conditions of the test.

In an *in vitro* chromosome aberration study in mammalian cells (Anonymous 32, 1994), Chinese hamster lung cells were treated with 1-nitropropane. Four treatment regimens were used: 6h treatment without metabolic activation (625, 1250, 2500 and 5000 µg/mL), 24 h treatment without metabolic activation (312.5, 625, 1250 and 5000 µg/mL), 48 h treatment without metabolic activation (312.5, 625, 1250 and 5000 µg/mL) and 6 h treatment with metabolic activation (156.25, 312.5, 625, 1250, 2500 and 5000 µg/mL).

No significant increase in the frequency of cells with chromosome aberrations was observed either in the presence or absence of a metabolic fraction at any of the exposure times. (see Table 15)

Table 15: 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 aberrations**	**Conc.**	**Cells with aberrations**	**Conc.**	**Cells with aberrations**	**Conc.**	**Cells with aberrations**
NC	4/200	NC	3/200	NC	2/200	NC	2/200
312.5	Not Eval.	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; Not Eval.: not evaluated; NC: negative control; MMC: mitomycine C; CP: cyclophosphamide

Consequently, it can be concluded that 1-nitropropane is not clastogenic to CHL cells *in vitro*.

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.

In an *in vitro* DNA damage and/or repair study (Andrae *et al.*, 1988), primary hepatocytes obtained from male and female Wistar rats were treated with 1-nitropropane.

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 %).

An *in vitro* gene mutation test in mammalian cells (Roscher *et al.,* 1990) was performed using Chinese hamster lung cells. Cells were treated with 1-nitropropane at a concentration of 0, 0.3, 1, 3, 6 and 10 mM during 3 h.

Marginal cytotoxicity was observed, the relative percent survival was approximately 95 % at 0.3 and 1 mM and 80 % at 3 and 10 mM.

1-nitropropane induced a higher number of TG (6-thioguanine) resistant mutants. The mutation frequency was approximetaly of 11, 18, 31, 53 and 46 $x10^6$ respectively at 0, 0.3, 1, 3 and 10 mM.

However, 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.

In an *in vitro* micronucleus test in mammalian cells (Roscher *et al.,* 1990), chinese hamster lung cells were exposed to 1-nitropropane at a concentration of 0, 0.3, 1, 3, 6 and 10 mM.

Marginal cytotoxicity was observed, the relative percent survival was approximetaly 95 % at 0.3 and 1 mM and 80 % at 3 and 10 mM.

1-nitropropane induced an increased number of micronuclei cells of 8, 6, 14 and 43 $x10^3$, respectively at 0, 1, 3 and 10 mM.

Nonetheless, 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.

An *in vitro* DNA damage and/or repair study (Andrae *et al.*, 1988) was performed and revealed that 1-nitropropane did not induce a DNA repair above control values in non-hepatic cell lines from rats, mouse, hamster and human.

In an *in vitro* gene mutation test in bacteria (Anonymous 33, 1994), 4 *S. Typh.* strains (TA98, TA100, TA1535 and TA1537) and *E. Coli* WP2uvrA- were treated with 1-nitropropane with and without metabolic activation. 2 independent experiments were performed using dose concentrations of 0, 8, 40, 200, 1000 and 5000 µg/plate for the first experiment and 0, 312.5, 625, 1250, 2500 and 5000 µg/plate for the second experiment.

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 (see Table 16 and Table 17).

Table 16: Number of revertants (number of colonies/plate) (experiment 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 17: Number of revertants (number of colonies/plate) (experiment 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

Under the test conditions, the compound is therefore considered as non-mutagenic.

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.

An *in vitro* gene mutation study in bacteria (Haworth *et al.*, 1983) was performed using 4 *S. Typh.* strains (TA98, TA100, TA1535 and TA1537).

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.

However, 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.

***In vitro* data on Nitromethane**

In an *in vitro* gene mutation test in bacteria (Mortelmans *et al.*, 1986), nitromethane was tested up to 10 mg/plate on 4 *S. typh.* strains (TA98, TA100, TA1535 and TA1537). Doses were chosen as 100, 333.3, 1000, 3333.3 and 10 000 µg/plate. 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.

Positive controls:

Strain	Without met. act.	With met. act.
TA98	4-nitro-o-phenylenediamine	2-aminoanthracene
TA100	sodium azide	2-aminoanthracene
TA1535	sodium azide	2-aminoanthracene

TA1537	9-aminoacridine	2-aminoanthracene

Overall, no significant increase in the frequency of revertant colonies was observed for any of the bacterial strains at 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 18: Ames test results

Dose level (µg/plate)		0	100	333.3	1000	3333.3	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

As a 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.* (1986) that only the last experimental results are presented in the article. However, DS 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?

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

Remarks: The full study report was not made available to the dossier submitter, the reliability of the study was therefore downgraded to 4 considering the low amount of data available. The data presented are extracted from the dissemination website or the IUCLID file.

In an *in vitro* chromosome aberration study in mammalian cells (NTP, 1997), nitromethane did not induce chromosomal aberration in CHO cells, either with and without metabolic activation, at concentrations as high as the limit concentration of 4980 µg/mL. It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentration the cells have actually been exposed.

Table 19: Chomosomal aberration in CHO cells

Compound	Dose level (µg/mL)	N cells	N aberrations	% cells with aberrations
Without met. act.				
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 met. act.				
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

In an *in vitro* sister chromatid exchange test in mammalian cells (NTP, 1997), nitromethane was unable to induce genotoxic effects on Chinese hamster ovary (CHO) cells via sister chromatid exchange mechanisms, both in the presence and in absence of metabolic activation, at concentration up to 4965 µg/mL. However, it is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentration cells have actually been exposed.

Table 20: SCE assay results in CHO cells

Dose level (µg/mL)		N cells	N chrom	N SCEs	SCE/chrom	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

In an *in vitro* gene mutation study in bacteria (Anonymous 28, 1975), results have to be taken with caution. Although not performed according to OECD TG 471, the overall quality of the test could be acceptable (dose-range finding, concurrent positive and negative controls, with and without metabolic activation,…), however, the compound was not soluble under treatment conditions, and consequently, it is not clear to which concentrations cells have been exposed. Furthermore, no specific 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 (swaps in reported results tables). The study was therefore disregarded due to poor data reporting.

In an *in vitro* gene mutation study in bacteria (Dayal *et al.*, 1989), nitromethane did not induce gene mutations in the absence of S9 mix, on 3 different strains of bacteria (TA98, TA100 and TA102). 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 the solution. As the reporting data are poorly reported, the study should nevertheless be interpreted with caution.

In an *in vitro* transformation study in mammalian cells (Kerckeart *et al.*, 1996), nitromethane induced a dose-dependent statistically significant increase in the morphological transformation frequency in SHE cells, in comparison with the negative control, at the two highest concentrations tested (4000 and 5000 µg/mL).

Table 21: SHE cells transformation test results

Dose level (µg/mL)	0	2000	2500	3000	3500	4000	5000
RPE (%)	100	86	86	92	84	84	76
N mutants	5	10	7	8	10	12	14
N total 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

As an *in vitro* micronucleus test performed in SHE cells was negative, the positive result observed in the SHE cells transformation test is probably induced by non-mutagenic mechanisms.

In an *in vitro* micronucleus test in SHE cells (Gibson *et al.*, 1997), nitromethane was incubated with SHE cells, the doses depending of the vehicle: 0 (DMSO), 5.0, 5.5 and 6.0 µg/mL and 0 (media), 3500, 4000, 5000 µg/mL. 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. The test results were negative, with either vehicle.

Table 22: SHE cells micronucleus test results with nitromethane

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

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-Hughes *et al.*, 1999) is considered to be limited and results were negative, the study was not included in this 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* data on Nitroethane**

In an *in vitro* gene mutation test (Mortelmans *et al.,* 1986), 4 bacterial *S. typh.* strains (TA98, TA100, TA1535 and TA1537) were exposed to nitroethane at doses of either 100, 333.3, 1000, 3333.3 or 10 000 µg/plate. No cytotoxicity was seen in any plate, except at the highest dose, in all strains. Precipitation was observed in the highest concentration tested in most experiments in all the strains. 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. 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 23: Ames test results

Dose level (µg/plate)		**0**	**100**	**333.3**	**1000**	**3333**	**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

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.

In another *in vitro* gene mutation test in bacteria (Anonymous 29, 1980), 5 strains of *S. typh.* (TA98, TA100, TA1535, TA1537 and TA1538) were exposed to vapours of nitroethane. A concentration of 55450 ppm caused cytotoxicity in strains TA1535 and TA1537 and therefore a concentration of 27725 ppm was tested. 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.

In a *in vitro* gene mutation test in mammalian cells report (Anonymous 30, 2012), results of 3 assays were provided. In the first one (preliminary) doses of either 0, 2.9, 5.9, 11.7, 23.5, 46.9, 93.9, 187.8, 375.5 or 751 µg/mL (= 10mM= limit dose) were selected. In the second and third tests (initial and confirmatory mutagenic tests, respectively), CHO cells were exposed to either 0, 46.9, 93.9, 187.8, 375.5, or 751 µg/mL. All tests were conducted with (+) and without (-) metabolic activation (S9). Positive controls were ethylmethanesulfonate (621 µg/mL) and 20-methylcholanthrene (4 and 8 µg/mL), for tests -S9 and +S9, respectively. No cytotoxicity was observed up to the highest concentration tested.

The preliminary test was run in triplicates and showed that 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 24: CHO cells survival (N colonies/plate) after exposure to NE 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

In the 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 25: 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 26: 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.

In the 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 27: 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	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 28: 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 29: HCD 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 *in vitro* mammalian gene mutation test at doses up to the limit concentration.

In an *in vitro* gene mutation study in bacteria (Dayal *et al*., 1989), 3 strains of *S. typh.* (TA98, TA100 and TA102) were exposed to nitroethane at concentrations under 200 µmol/plate. Nitroethane was negative in the *in vitro* gene mutation tests but they were only performed in 3 bacterial strains and in absence of S9 metabolic fraction. 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, in the same study, 2-nitropropane induced a positive result at a low concentration (20 µmol/plate) suggesting that the test material remained in solution.

***In vivo* data on 1-Nitropropane**

In an *in vivo* micronucleus test (George *et al.*, 1989), groups of 4 to 8 male SD rats were exposed by gavage to a single dose of 1-nitropropane. Animals were sacrificed 24 or 48 h (bone marrow) or 72 h (liver) after dosing.

Regarding the bone marrow test, after treatment with 1-nitropropane (experiment A), a slight lower percentage of polychromatic erythrocytes (PCE) was observed as well as a slight dose-related increase in the frequency of micronucleated cells compared to control. Since no sign of toxicity were observed in the first experiment, a second experiment was performed and did not exhibit cytotoxicity or an increased frequency of micronucleated cells (see Table 30).

Table 30: 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[A]	8.40[A]	0.92	1.17	1.08	1.83	1.33	1.70	1.50	8.33[A]
% 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

[A]: $p<0.05$; 2000 PCE analysed for micronucleus frequency; 500 erythrocytes for %

Regarding liver cell test, a higher frequency of micronuclei in hepatocytes was observed. 17.05 micronucleated cells/1000 hepatocytes in treated animals was noted compared to 7.34 micronucleated cells/1000 hepatocytes in control group. This effect was accompanied by an increased mitotic index (28.85 mitoses/1000 hepatocytes vs 14.92 mitoses/1000 hepatocytes). Furthermore, in a second experiment, 14.20 micronucleated cells/1000 hepatocytes in treated animals were observed compared to 5.03 micronucleated cells/1000 hepatocytes.

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.

Nonetheless, based on the available data, it is not clear whether 1-nitropropane reached the bone marrow.

In an *in vivo* mammalian cell study, DNA damage and/or repair (Andrae *et al.,* 1988), Wistar rats were exposed by intraperitoneal exposure to 1-nitropropane at a concentration of 0, 20, 40, 60 and 80 mg/kg.

The article mentions that “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*"

An *in vivo* mammalian somatic cell study, cytogenicity/erythrocyte micronucleus (Kliesch and Adler, 1987) was performed in mouse. 5 males and 5 females per group were exposed to a single intraperitoneal injection to 1-nitropropane.

No dose or time-dependent increase in the frequency of micronucleated polychromatic erythrocyte was observed.

***In vivo* data on Nitromethane**

In an *in vivo* micronucleus test (NTP, 1997) in B6C3F1 mouse normochromatic erythrocytes, no increase in the frequencies of micronucleated erythrocytes was observed in the peripheral blood of male or female mice that had been exposed to nitromethane by inhalation for 13 weeks at concentrations up to 1500 ppm. 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 effect was observed in the *in vitro* chromosome aberration and micronucleus test.

Gocke *et al.* (*in vivo* micronucleus test, 1981) study 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.

***In vivo* data on Nitroethane**

In an *in vivo* micronucleus test (Hite and Skeggs, 1979), 8 CD-1 mice per sex (14 in controls) were exposed to either 0, 0.25, 0.50 or 1 mL/kg bw/d nitroethane by oral gavage, in two doses each day. 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.

Table 31: Percentage of polychromatic erythrocytes with micronuclei, in %

Dose level (mL/kg bw/d)		0 (tap water)	0.25	0.50	1	Positive control
Exposure route		p.o.	p.o.	p.o.	p.o.	IP
Sex	Male	0.53	0.51	0.67	0.60	5.76 ***
	Female	0.64	0.44	0.47	0.57	6.09 ***
	Combined	0.58	0.48	0.57	0.59	5.92 ***

***: $p < 0.001$

Based on the available information, it is however 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.

10.8.2 Comparison with the CLP criteria

CLP criteria cat. 1	CLP criteria cat. 2
Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans. The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1B is based on: – positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or – positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or – positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: – Positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: – Somatic cell mutagenicity tests *in vivo*, in mammals; or – Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

Mutagenic tests on 1-Nitropropane were negative in several bacterial gene mutations tests (Anonymous 31, 1996; Anonymous 32, 1994; Haworth *et al.*, 1983).

A non significant increase in the number of 6-thioguanine resistant mutations was observed in Chinese Hamster lung cells V79 after treatment with 1-nitropropane (Roscher *et al.*, 1990). However, 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.

Furthermore, whereas an *in vitro* chromosome aberration test in Chinese Hamster Lung cells was clearly negative with and without metabolic activation (Anonymous 33, 1994), an increased formation of micronuclei in Chinese Hamster lung cells V79 treated with 1-nitropropane in absence of metabolic fraction was observed in another study (Roscher *et al.*, 1990).

Positive results of 1-nitropropane in an *in vitro* unschedulded DNA synthesis (UDS) assay (Andrae *et al.*, 1988) were also provided by the applicant. However, these data should be considered with caution as the *in vitro* UDS test method is considered obsolete and has been deleted from the OECD TG program.

Finally, 1-Nitropropane was negative in an *in vivo* micronucleus test (George *et al.*, 1989) in bone marrow but positive in a liver micronucleus test.

Furthermore, all *in vitro* tests (both key and supporting studies) with nitromethane addressing gene mutations (in bacteria) and chromosome aberrations were negative. For some tests, it was unclear whether the protocol was adapted for volatile compounds. However, overall, cells have been exposed to sufficiently high concentrations of nitromethane.

No data of gene mutation studies in mammalian cells with nitromethane were provided but read-across with the results of nitroethane in an *in vitro* Chinese hamster ovary cell/hypoxanthineguanine-phosphoribosyl transferase (CHO/hgprt) forward gene mutation study was performed. Based on the outcome of the read-across, nitromethane was also considered to be negative for gene mutations in mammalian cells.

Nitromethane was also negative in two (one key and one supporting) *in vivo* micronucleus studies. Although it was not clear whether the substance reached the bone marrow in these studies, the compound was tested in high concentrations, and together with the lack of effect of nitromethane in the *in vitro* chromosome aberration, this may be sufficient. A positive result was only obtained in the SHE transformation assay. As this test responds to different mechanisms including non-mutagenic mechanisms, this outcome does not provide evidende for mutagenicity.

Moreover, all *in vitro* tests with nitroethane addressing gene mutations (in bacteria and mammalian cells) were clearly negative. Although for some tests it was unclear whether the protocol was adapted for volatile compounds, in two key studies (1 bacterial and 1 mammalian) special precautions were taken for working with this type of compound.

No data from *in vitro* chromosome aberration tests and/or micronucleus tests were provided. To address the endpoint of structural and numerical chromosome aberrations, data of an *in vivo* micronucleus test were used. Nitroethane did not induce a statistically significant incease in the micronucleus frequency at any of the doses tested. However, based on the available information, it was unclear whether nitroethane reached the bone marrow. Consequently, the negative result of the *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.

Table 32: Summary data regarding *in vitro* tests

In vitro				
Test Guidelines	**Substances**	**Results**	**References**	**Remarks**
OECD TG 471	NM	Negative	Mortelmans *et al.*, 1986	/
	NM	Negative without S9	Dayal *et al.*, 1989	/
	NM	Negative	Anonymous 27, 1980	Prior to an OECD TG 471 test
	NM	-	Anonymous 28, 1975	Prior to an OECD TG 471 test Disregarded due to poor data reporting + test material not soluble under the treatment conditions
	NE	Negative	Mortelmans *et al.*, 1986	/
	NE	Negative without	Dayal *et al.*, 1989	/

		S9		
	NE	Negative	Anonymous 29, 1980	Prior to an OECD TG 471 test
	1-NP	Negative	Anonymous 31, 1996	/
	1-NP	Negative	Anonymous 32, 1994	/
	1-NP	Negative	Haworth *et al.*, 1983	/
OECD TG 473	NM	Negative	NTP, 1997	/
OECD TG 476	NE	Negative	Anonymous 30, 2012	/
	1-NP	Positive	Roscher *et al.*, 1990	Cytotoxicity : yes
	1-NP	Negative	Andrae *et al.*, 1988	/
OECD TG 479	NM	Negative	NTP, 1997	/
OECD TG 482	1-NP	Negative	Andrae *et al.*, 1988	/
OECD TG 487	1-NP	Positive	Roscher *et al.*, 1990	Cytotoxicity: yes
EU method B.21	NM	Positive	Kerckaert *et al.*, 1996	/
No guideline - micronucleus test in SHE cells	NM	Negative	Gibson *et al.*, 1997	/
No guideline - chromosome aberration study in mammalian cells	1-NP	Negative	Anonymous 33, 1994	/

Table 33: Summary data regarding *in vivo* tests

In vivo				
Test Guidelines	**Substances**	**Results**	**References**	**Remarks**
OECD TG 474	NM	Negative	NTP, 1997	/
	NE	Negative	Hite and Skeggs, 1979	/
No guideline micronucleus test	1-NP	Negative in the bone marrow Positive in the liver	George *et al.*, 1989	/
No guideline mammalian cell study : DNA damage and/or repair	1-NP	Negative	Andrae *et al.*, 1988	/

No guideline mammalian somatic cell study: cytogenicity/erythrocyte micronucleus	1-NP	Negative	Kliesch and Adler, 1987	/

In conclusion, no evidence for classification of nitromethane, nitroethane and 1-nitropropane for germ cell mutagenicity was found in the reported studies. The DS notes however that the metabolism of nitromethane leads to the formation of formaldehyde which has a harmonised classification as Muta. 2, H341.

For many of the *in vitro* tests, it was not indicated whether the protocol had been adapted for volatile compounds and, consequently, it remains unknown to which concentrations cells have actually been exposed.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the information provided by the applicant, there is no evidence for classification of nitromethane and nitroethane for germ cell mutagenicity. However, data are insufficient to allow characterization of the complete mutagenic profile of the compound.

Although 1-nitropropane was non-mutagenic in bacteria and did not cause structural chromosome aberrations in CHL cells, positive results were reported in some other *in vitro* genotoxicity tests. Furthermore, with respect to the *in vivo* micronucleus test, it should be noted that no guideline was used to design the study and no raw data was made available to the DS. The validity of the study remains therefore uncertain and the reliability, as well as the relevance of the available results for classification, are considered as low.

Consequently, data is considered inconclusive for germ cell mutagenicity.

10.9 Carcinogenicity

Table 34: Summary table of animal studies on carcinogenicity

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
1-NITROPROPANE			
Long term inhalation toxicity study Rat / Long Evans / male + female 125/sex (10/sex/group and the remaining alive were killed after 21.5 months of exposure) No guideline followed GLP compliance: unspecified Reliability 2 (according to the registration dossier)	**1-nitropropane** Purity: unspecified Doses: 0 or 100 ppm, approx. equivalent to 0 and 0.369 mg/L, resp Duration of exposure: 1, 3, 12, 18 and 21.5 months + 2 additional groups: exposed during 21.5 months and thereafter observed during 3 months or 12 months	Mortality: increased in treated groups Clinical signs: not specified Body weight: inconsistent differences, no treatment-related effects Organ weight: no treatment-related changes (brain, kidneys, liver examined) Histopathology: few incidences of liver vacuolization and a number of parenchymal abscesses in animals found dead Benign tumours: increased incidence of pituitary adenoma after 18 m of exposure (in control and treated groups) Malignant tumours: slightly increased incidence of lymphosarcoma in spleen and lymph nodes in animals found dead in control and treated groups	Griffin *et al.*, 1982
Assay of 1-nitropropane, 2-nitropropane, 1-azoxypropane and 2-azoxypropane for carcinogenicity Rat / SD / male Nb of animals not specified Gavage No guideline followed Not-GLP Reliability 2 (according to the registration dossier, however only summary available to the DS)	**1-nitropropane** Doses: 0 and 89.1 mg/kg bw 3 times/week for 16 w followed by 1 time/w for 10 w Duration of exposure: 26 w Surviving animals were sacrificed after 77 w	Body weight and necropsy findings: treatment-related effects observed (no more information available) No increase of tumour incidence (no more detail given)	Fiala *et al.,* 1987

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Test for chemical carcinogens Rat / F344 / both sexes Nb: 3/sex/dose except at the mid-dose (15/sex) Gavage No guideline reported Not-GLP No access to raw data, not reported in the registration dossier	**1-nitropropane** Doses: 0, 0.3, 3 or 10 mg/d 5 times/week, for 52 weeks	No increase in tumour incidence	Hadidian *et al*., 1968
NITROMETHANE			
Long term inhalation toxicity study Similar to OECD TG 451 GLP-compliant 2 years Rats / F344 / both sexes 50/sex/dose Reliability 1 (according to the registration dossier)	**Nitromethane** Purity: > 99 % Inhalation 6 h/d, 5 d/w 0, 94, 188, 375 ppm (approx. equivalent to 0, 0.235, 0.47 and 0.94 mg/L, resp.)	Mortality: relatively high in all groups but not dose-related (74, 68, 72 and 84 % in males and 44, 62, 40 and 54 % in females at 0, 94, 188 and 375 ppm, resp.) Clinical signs: masses on shoulders and torso consistent with mammary gland neoplasms BWG: slightly increased in females exposed to 375 ppm vs. controls Organ weight: no data Histopathology: - In males: hyperplasia in renal tubule (6, 8, 6 and 12 out of 50 males, at 0, 94, 188 and 375 ppm, resp.) - In females: mammary gland fibroadenoma, fibroadenoma or adenoma (combined) and fibroadenoma, adenoma or carcinoma (combined) increased in a dose-dependent manner (see below) Neoplastic effects: In females: **Mammary gland**, out of 50 animals and at 0, 94, 188 and 375, resp. (%):	NTP, 1997

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		- Adenoma: 2 (4), 0 (0), 0 (0), 2 (4) (HCD: 0-4 %) - Fibroadenoma: 19 (38), 21 (42), 33 (66)*, 36 (72)* (HCD: 20-40 %) - Carcinoma: 2 (4), 7 (14), 1 (2), 11 (22)* (HCD: 0-8 %) - Adenoma, fibroadenoma or carcinoma: 21 (42), 25 (50), 35 (68)*, 41 (82)* (HCD: 22-46 %)	
Long term inhalation toxicity study Equivalent or similar to OECD TG 451 GLP-compliant 2 years Mice / B6C3F1 / both sexes 50/sex/group Reliability 1 (according to the registration dossier)	**Nitromethane** Purity: > 99 % Impurities: 0.25 % nitroethane, 0.03 % 2-nitropropane Inhalation 6 h/d, 5 d/week 0, 188, 375, 750 ppm (approx. equivalent to 0, 0.47, 0.94 and 1.87 mg/L, resp.)	Mortality: 38, 28, 40 and 42 % of males and 50, 44, 48 and 28 % of females exposed to 0, 188, 375 and 750 ppm, resp., died Clinical sign: in the eyes, swelling and exophthalmos coincident with harderian gland tumours, in both sexes BWG: no effects in males, slightly increased BW in females during the study but similar to controls at study termination Organ weights: no data Histopathology: - Sign. increased incidence olfactory epithelium degeneration in both sexes, in all treated groups - Sign. increase in olfactory epithelium metaplasia in both sexes at 375 and 750 ppm - Sign. increase in respiratory epithelium hyaline degeneration in all treated groups in females and at the middle and high doses in males. Neoplastic effects **- Harderian gland**: Male and female: Adenoma (%): M: 9/50 (18), 10/50 (20), 19/50 (38)**, 32/50 (64)** (HCD: 2-14 %) F: 5/50 (10), 7/50 (14), 16/50 (32)**, 19/50 (38)** (HCD: 0-16 %) Carcinoma (%): M: 1/50(2), 1/50 (2), 6/50 (12), 5/50 (10) (HCD: 0-4 %) F: 1/50 (2), 2/50 (4), 4/50 (8), 3/50 (6) (HCD: 0-4 %)	NTP, 1997

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Adenoma or carcinoma (%): M: 10/5 (20), 11/50 (22), 25/50 (50)**, 37/50 (74)** (HCD: 2-14 %) F: 6/50 (12), 9/50 (18), 20/50 (40)**, 21/50 (42)** (HCD: 0-16 %) **- Liver**: Female (%): Hepatocellular adenoma: F: 14/50 (28), 25/49 (51)*, 17/49 (35), 35/50 (70)** (HCD: 0-40 %) Hepatocellular carcinoma: F: 10/50 (20), 14/49 (29), 8/49 (16), 12/50 (24) (HCD: 2-30 %) Hepatocellular adenoma or carcinoma: F: 19/50 (48), 34/49 (69)**, 22/49 (45), 40/50 (80)** (HCD: 6-54 %) No increase in liver tumours was observed in Males. **Lung:** Male and female (%): Alveolar/bronchiolar adenoma: M: 11/50 (22), 10/50 (20), 9/50 (18), 12/50 (24) (HCD: 6-36 %) F: 3/50 (6), 3/50 (6), 2/49 (4), 9/50 (18) (HCD: 0-14 %) Alveolar/bronchiolar carcinoma: M: 2/50 (4), 3/50 (6), 3/50 (6), 11/50 (22)** (HCD: 0-16 %) F: 0/50 (0), 3/50 (6), 5/49 (10), 3/50 (6) (HCD: 0-6 %) Alveolar/bronchiolar adenoma or carcinoma: M: 13/50 (26), 13/50 (26), 12/50 (24), 20/50 (40) (HCD: 10-42 %) F: 3/50 (6), 6/50 (12), 6/49 (12), 12/50 (24)* (HCD: 0-16 %)	
Long term inhalation toxicity study Rats / Long-Evans / male + female	**Nitromethane** Purity: 96.26 % Impurities: 2.79 % nitroethane,	Mortality: 37.5, 42.5 and 37.5 % of males and 25, 27.5 and 40 % of females died Body weights: - similar to controls in males, - sign. lower than controls in females after 1 year exposure at 100 and 200	Anonymous 34, 1990

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
40 animals/group 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,…)	0.62 % 2-nitropropane Inhalation Doses: 0, 100, 200 ppm (approx. equivalent to 0, 0.25 and 0.50 mg/L, resp.) Duration of exposure: 7 h/d, 5 d/w for 103 w	ppm Clinical chemistry: no clinically significant effects in either sex Hematology: no effects in either sex Organ weights (brain, liver, kidneys, lungs, heart): no effects in relative and absolute weights, in both sexes Histopathology: 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. Neoplastic effects: - No treatment-related increase in tumours incidence. - In all animals, benign tumours (adenoma of the pituitary gland, fibroadenomas and multiple fibroadenomas of the mammary glands) were observed but the incidence was similar in control and exposed animals, in both sexes. - Malign tumours were very rare and no treatment-relationship was observed.	
NITROETHANE			
Long term inhalataion toxicity study 2 years Similar to OECD TG 453 GLP compliant: not specified Rat Long-Evans 40/group (control & 100 ppm) 41 males & 39 females (200 ppm) Reliability 2 (according to the	**Nitroethane** Purity: 97.92 % Impurities: nitromethane 0.01 % and 2-nitropropane 2.07 % Inhalation 7 h/d, 5 d/w Conc.: 0, 100, 200 ppm (corresp. approx. to 0, 0.31 and 0.61 mg/L, resp.)	Mortality: no treatment-related effect BW: sign. ↓ at 100 ppm in males and at 200 ppm in females Clinical chemistry: slight but sign. ↑ of tot. prot. and BUN in females exposed to 200 ppm Hematology: No effects observed. MetHb levels not assessed. Organ weights (brain, liver, kidneys, lungs, heart): no treatment-related effect Histopathology: no effect Neoplastic effects: - No treatment-related increase of tumours	Anonymous 35, 1986

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
registration dossier) Major deviations: - only 2 doses tested - 40 animals / group - some tissues were not examined microscopically (parathyroid, caecum, rectum, bone marrow,…)		- In all animals (controls and treated groups), high incidence of benign tumours (adenoma of the pituitary gland) - Very rare malign tumours, not treatment-related - No HCD available	

No human data or other relevant information available.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Data on 1-Nitropropane

In an long-term inhalation toxicity study (Griffin *et al.*, 1982), 125 male and 125 female rats were exposed to 1-nitropropane at a concentration of 0 or 100 ppm (approximatively equivalent to 0 and 0.369 mg/L, respectively). Groups of rats (10/sex/group) were exposed and sacrificed either after 1, 3, 12 or 18 months of exposure. Additional recovery groups (10/sex/group) were removed from the exposure chamber after 3 and 12 months and thereafter were non-exposed until the end of the study period. All remaining alive animals were killed after 21.5 months.

Inconsistent differences were observed during the body weight and hematology examination (see Table 35 and Table 36). Necropsy did not reveal any treatment-related organ weight changes, and only infrequent findings were observed amongst control and exposed groups.

Table 35: Body weight data (in g)

	Males		Females	
	0 ppm	100 ppm	0 ppm	100 ppm
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)[a]	/	381 (4)[a]
12 m + 9.5 m of recovery	/	636 (6)[a]	/	357 (8)[a]

(): nb of animals examined, [a]: compared to 21.5 m controls

Table 36: Methemoglobin (in mg/dL)

	Males		Females	
	0 ppm	100 ppm	0 ppm	100 ppm
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)[a]	/	19 (3)[a]
12 m + 9.5 m of recovery	/	43 (6)[a]	/	50 (8)[a]

(): nb of animals examined; [A]: DS's remarks: 12 animals noted in the full study report while 10 animals in the group; [a]: compared to 21.5 m controls

Regarding the histopathology, an increased incidence of pituitary adenoma was observed after 18 months and an increased incidence of islet adenoma was noted at the end of the study, however these incidences were similar in the control and exposed groups (see Table 37 and Table 38). The most common malignant

tumour was lymphosarcoma in spleen and lymph nodes after 18 months, however as the benign tumour, the incidence was similar in control and treated groups (see Table 39 and Table 40).

Table 37: Incidence (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	5/19
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		

Table 38: Incidence (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		

Table 39: Incidence (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 40: Incidence (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

An assay of 1-nitropropane, 2-nitropropane, 1-azoxypropane and 2-azoxypropane for carcinogenicity was performed by gavage in Sprague-Dawley rats (Fiala *et al.*, 1987). Animals were exposed to 0 or 89.1 mg/kg bw/day, 3 times per week for 16 weeks, followed by 1 time per week for 10 weeks. Surviving animals (26) were sacrificed and necropsied after 77 weeks. Body weight and necropsy examination revealed treatment-related effects (no more information available). The histopathology did not show an increase in tumour incidence (no more information available).

In a test for chemical carcinogens (Hadidian *et al.*, 1968; Report on the activity of derivatives of aromatic amines, nitrosamines, quinolines, nitroalkanes, amides, epoxides, aziridines, and purine antimetabolites), animals were exposed to 1-nitropropane 5 times a week for a year to either 0, 0.3, 3 or 10 mg/day. No increase in tumour was reported. No more information is available either on species, final exposure dose or effects.

Data on Nitromethane

In a long term inalahation toxicity study in rats (NTP, 1997), male and female Fisher F344/N rats were exposed during 2 years to vapours of nitromethane at doses of either 0, 94, 188 or 375 ppm (6 h/d, 5 d/w). The doses of 0, 94, 188 and 375 ppm were approximatively equivalent to 0, 0.235, 0.47 and 0.94 mg/L, respectively. Mortality was relatively high in all dose groups, in both sexes, but was not dose-related.

Table 41: 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)

Body weights were not affected in males but they were slightly higher than in controls in females exposed to 375 ppm.

Table 42: 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)

Masses on shoulders 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.

At necropsy, in females, the incidences of fibroadenoma, fibroadenoma or adenoma (combined) and of fibroadenoma, adenoma or carcinoma (combined) of the mammary gland increased in a dose-dependent manner (as observed in Table 43), confirming clinical observations and possibly explaining the increase in body weights at higher doses.

Table 43: Incidence of tumours in males and females rats

Dose exposure level (ppm)		0	94	188	375	HCD[a] Tot. (% ± St. Dev.) Range
Males	No tumours reported					
Females	Adenoma (%)	2/50 (4)	0/50	0/50	2/50 (4)	3/348 (0.9 ± 1.6 %) 0-4 %
	Fibroadenoma (%)	19/50 (38)	21/50 (42)	33/50** (66)	36/50** (72)	97/348 (27.9 ± 7.3 %) 20-40 %
	Carcinoma (%)	2/50 (4)	7/50 (14)	1/50 (2)	11/50** (22)	14/348 (4 ± 2.6 %) 0-8 %
	Adenoma, fibroadenoma and carcinoma (%)	21/50 (42)	25/50 (50)	35/50** (70)	41/50** (82)	108/348 (30.9 ± 9.1 %) 22-46 %

[a]: HCD of mammary gland neoplasms incidence at Battelle Pacific Northwest Laboratories, in F344/N female rats, 1995; * shows statistical significance with the Fisher exact test $p<0.05$ and **$p<0.01$

In female rats, the incidence of fibroadenoma, fibroadenoma or adenoma, and fibroadenoma, adenoma or carcinoma was dose-dependent and incidences at the middle and high doses were statistically significant. The tumours incidence in the low, mid and high dose groups were outside the range of the historical control data, whereas, incidence in control group was included in these ranges. Carcinomas tended to appead earlier in treated groups, compared to the control group.

Table 44: 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

Table 45: 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**

In bold, statistically significant

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 control and exposed groups. The logistic regression test regards neoplasms in animals as nonlethal. A lower incidence in an exposed group is indicated by N.

In a long term toxicity study in mice (NTP, 1997), male and female B6F3C1 mice were exposed during 2 years to vapours of nitromethane at doses of either 0, 188, 375 or 750 ppm (6 h/d, 5 d/week). The doses of 0, 188, 375 and 750 ppm were approximatively equivalent to 0, 0.47, 0.94 and 1.87 mg/L, respectively. Mortality tended to be high in all dose groups (see Table 46), in both sexes, but the survival rate of females exposed to the highest dose was marginally greater than in other groups. Coincidently with a swelling around the eyes and exophthalmos in exposed animals of both sexes, neoplasms of the Harderian gland were observed (see Table 49 below). Nasal lesions were reported in a great number of exposed animals of both sexes (see Table 48). Tumours incidence in the Harderian gland, the liver and the lung are presented in Table 49 below. Liver tumours were seen only in females.

Table 46: Mortality rate in male and female mice exposed by inhalation to NM

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)

Body weight gains were not affected by the treatment in males. In females, mean BW were similar in all dose groups at study termination.

Table 47: Mean BW (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

In both sexes, swelling around the eyes and exophthalmos were reported. These effects were coincident with harderian gland neoplasms.

Histopathological findings show that nasal lesions were increased in exposed animals. Nasolacrimal duct inflammation was reported in 2, 3, 10 and 10 males and 1, 0, 3 and 3 females respectively exposed to 0, 188, 375 and 750 ppm.

Table 48: Histopathological findings in mice

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

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

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. No similar tissue is found in humans.

For the liver tumours, only observed 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 well.

Table 49: Tumours incidence in the Harderian gland, the liver and the lung of mice exposed for 2 years by inhalation to NM

Dose level exposure (ppm)			0	188	375	750	HCD[a] Tot. (% ± St. Dev.) Range
Harderian Gland	Adenoma	M (%)	9/50 (18)	10/50 (20)	19/50 (38)*	32/50 (65)**	36/450 (8 ± 4.2 %) 2-14 %
		F (%)	5/50 (10)	7/50 (14)	16/50 (32)**	19/50 (38)**	21/447 (4.7 ± 5.0 %)

							0-16 %
	Carcinoma	M (%)	1/50 (2)	1/50 (2)	6/50 (12)	5/50 (10)	2/450 (0.4 ± 1.3 %) 0-4 %
		F (%)	1/50 (2)	2/50 (4)	4/50 (8)	3/50 (6)	6/447 (1.3 ± 1.7 %) 0-4 %
	Adenoma or carcinoma	M (%)	10/50 (20)	11/50 (22)	25/50 (50)**	37/50 (74)**	38/450 (8.4 ± 4.0 %) 2-14 %
		F (%)	6/50 (12)	9/50 (18)	20/50 (40)**	21/50 (42)**	27/447 (6.0 ± 5.0 %) 0-16 %
Liver	Hepatocellular adenoma	M (%)	No effects reported				-
		F (%)	14/50 (28)	25/49 (51)**	17/49 (35)	35/50 (70)**	51/446 (11.4 ± 12.4 %) 0-40 %
	Hepatocellular carcinoma	M (%)	No effects reported				-
		F (%)	10/50 (20)	14/49 (29)	8/49 (16)	12/50 (24)	54/446 (12.1 ± 8.1 %) 2-30 %
	Hepatocellular adenoma or carcinoma	M (%)	No effects reported				-
		F (%)	19/50 (38)	34/49 (69)**	22/49 (45)	40/50 (80)**	95/446 (21.3 ± 14.8 %) 6-54 %
Lung	Alv/bronch adenoma	M (%)	11/50 (22)	10/50 (20)	9/50 (18)	12/50 (24)	76/448 (17 ± 8.7 %) 6-36 %
		F (%)	3/50 (6)	3/50 (6)	2/49 (4)	9/50 (18)	32/446 (7.2 ± 3.8 %) 0-14 %
	Alv/bronch carcinoma	M (%)	2/50 (4)	3/50 (6)	3/50 (6)	11/50 (22)**	37/448 (8.3 ± 5.8 %) 0-16 %
		F (%)	0/50 (0)	3/50 (6)	5/49 (10)**	3/50 (6)	15/446 (3.4 ± 2.4 %) 0-6 %
	Alv/bronch adenoma or	M (%)	13/50 (26)	13/50 (26)	12/50 (24)	20/50 (40)	108/448 (24.1 ± 9.5 %)

	carcinoma						10-42 %
		F (%)	3/50 (6)	6/50 (12)	6/49 (12)	12/50 (24)**	46/446 (10.3 ± 4.6 %) 0-16 %

[a]: Battelle Pacific Northwest laboratories, in B6C3F1 mice, 1995; Alv/Bronch = alveolar / bronchiolar

Table 50: 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

Table 51: 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**

In bold, statistically significant

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 52: 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**

In bold, statistically significant

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 53: 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**

In bold, statistically significant

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.

In a long term inhalation toxicity study (Anonymous 34, 1990), male and female Long-Evans rats were exposed to vapours of nitromethane at doses of either 0, 100 or 200 ppm for 2 years (0, 100 and 200 ppm were approximatively equivalent to 0, 0.25 and 0.50 mg/L, respectively). Mortality was unaffected by the treatment (Table 54 below). No clinical signs were reported. Body weights were similar in exposed and in control groups in males, but in females, it was significantly lower after 1 year of exposure at 100 and 200 ppm.

Table 54: Mortality rate

Dose exposure level (ppm)	0	100	200
Males (%)	15/40 (37.5)	17/40 (42.5)	15/40 (37.5)
Females (%)	10/40 (25)	11/40 (27.5)	16/40 (40)

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 in females, at 0, 100 and 200 ppm, respectively). For hematological parameters, no effects were reported on WBC, RBC, Hb, Ht, MCV, PLT counts after 2 years of exposure, in both sexes (see the Annex I for detailed data).

No effects were reported in either sex on absolute & relative brain, liver, kidneys, lungs and heart weights (see the Annex I for detailed data).

Histopathological findings were observed in all animals (controls + exposed), but the effects were not treatment-related (bronchitis, glomerulosclerosis, calcification of the kidneys, vacuolation of the adrenal cortex and fibrocystomas in the mammary gland).

In all animals, benign tumours (adenoma of the pituitary gland, fibroadenomas and multiple fibroadenomas of the mammary glands) were observed but the incidence was similar in control and exposed animals. Malign tumours were very rare and no treatment-relationship was observed.

Table 55: 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	Metastasis Primary mesenchymal	0	2	1

Malign tumours in bold

Data on Nitroethane

In a long-term inhalation toxicity study (Anonymous 35, 1986), rats were exposed during 2 years to either 0, 100 or 200 ppm nitroethane by inhalation. Mortality was relatively high in all dose groups, without any dose-response relationship. Indeed, as showed in Table 56 below, at least 50 % of the control group did not survive during the 2-year study. No historical control data is available.

Table 56: Mortality rate

Dose level (ppm)	0	100	200
Male (%)	20/40 (50)	21/40 (52.5)	17/41 (41.5)
Female (%)	23/40 (57.5)	23/40 (57.5)	14/39 (35.9)

Body weights were significantly decreased at 100 ppm in males and at 200 ppm in females, 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.

No relevant effects were reported after clinical chemistry and haematology data assessment. Organ weights were not affected by the treatment. Concerning histopathology, no other effects than usual age-associated degenerative diseases and the endocrine target organ response to pituitary hyperplasia were observed and they were similar in controls and exposed animals.

No treatment-related increase of tumours was observed in either dose group. Incidence of benign tumours (adenoma of the pituitary gland) was high in control and treated groups. Very rare malignant tumours were seen in mammary gland, salivary gland, liver and kidney.

Table 57: Neoplastic findings incidence in pituitary gland (%)

Concentration levels (ppm)		0	100	200
Nodular hyperplasia	M	13/38 (34)	15/39 (38)	15/40 (38)
	F	7/38 (1)	6/40 (15)	12/37 (32)
Adenoma	M	22/38 (58)	16/39 (41)	16/40 (40)
	F	27/38 (71)	26/40 (62)	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)

In vivo				
Test Guidelines	**Substances**	**Results**	**References**	Remarks
Similar to OECD TG 451	NM	Increased incidence of neoplasia in mammary glands in females (%) - Fibroadenoma: 19 (38), 21 (42), 33 (66)*, 36 (72)* (HCD: 20-40 %) - Carcinoma: 2 (4), 7 (14), 1 (2), 11 (22)* (HCD: 0-8 %) - Adenoma, fibroadenoma or carcinoma: 21 (42), 25 (50), 35 (68)*, 41 (82)* (HCD: 22-46 %)	NTP, 1997	In rats High mortality in all dose groups, not dose-related, in both sexes
Similar to OECD TG 451	NM	Increased incidence of neoplasia in Harderian gland Increased incidence of neoplastic effects in females liver (%): Hepatocellular adenoma: F: 14/50 (28), 25/49 (51)*, 17/49 (35), 35/50 (70)** (HCD: 0-40 %) Hepatocellular carcinoma: F: 10/50 (20), 14/49 (29), 8/49 (16), 12/50 (24) (HCD: 2-30 %) Hepatocellular adenoma or carcinoma: F: 19/50 (48), 34/49 (69)**, 22/49 (45), 40/50 (80)** (HCD: 6-54 %) Increased incidence of neoplastic	NTP, 1997	In mice High mortality in all dose groups, not dose-related, in both sexes Effects in Harderian gland are not relevant for human health

		effects in the lung of both sexes Alveolar/bronchiolar adenoma: M: 11/50 (22), 10/50 (20), 9/50 (18), 12/50 (24) (HCD: 6-36 %) F: 3/50 (6), 3/50 (6), 2/49 (4), 9/50 (18) (HCD: 0-14 %) Alveolar/bronchiolar carcinoma: M: 2/50 (4), 3/50 (6), 3/50 (6), 11/50 (22)** (HCD: 0-16 %) F: 0/50 (0), 3/50 (6), 5/49 (10), 3/50 (6) (HCD: 0-6 %) Alveolar/bronchiolar adenoma or carcinoma: M: 13/50 (26), 13/50 (26), 12/50 (24), 20/50 (40) (HCD: 10-42 %) F: 3/50 (6), 6/50 (12), 6/49 (12), 12/50 (24)* (HCD: 0-16 %)		
OECD TG 451	NM	Non treatment-related effects in all animals: bronchitis, glomerulosclerosis, calcification of the kidneys, vacuolation of the adrenal cortex and fibrocystomas in the mammary gland No treatment-related increase in tumours incidence In all animals, benign tumours (adenoma of the pituitary gland, fibroadenomas and multiple fibroadenomas of the mammary glands), not treatment-related	Anonymous 34, 1990	/

		Very rare malign tumours, not treatment-related		
Similar to OECD TG 453	NE	No treatment-related increase of tumours Increased incidence of benign tumours (adenoma of the pituitary gland) in all animals Very rare malign tumours, not treatment-related	Anonymous 35, 1986	/
No guideline, 2-year inhalation	1-NP	Increased incidence of pituitary adenoma after 18m of exposure Slightly increased incidence of lymphosarcoma in spleen and lymph nodes in animals found dead in control and treated groups	Griffin *et al.*, 1982	/
No guideline, carcinogenicity study	1-NP	No increase of tumour incidence	Fiala *et al.,* 1987	/
No guideline, Test for chemical carcinogens	1-NP	No increase in tumour incidence	Hadidian *et al*., 1968	/

Table 58: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
1-NITROPROPANE								
Rat (Long-Evans)	Benign tumours: pituitary adenoma Malign tumours: lymphosarcoma in spleen and lymph nodes Tumours were observed in exposed and control groups	Yes	Yes	/	Both sexes	/	Inhalation	/
NITROMETHANE								
Rat (Long-Evans)	No treatment-related increase of tumours	/	/	/	/	/	Inhalation	/
Rat (F344)	**Mammary gland**: Adenoma, fibroadenoma or carcinoma	No	Yes	/	Only in females	/	Inhalation	Non-genotoxic but a positive result was obtained in the SHE transformation assay The concordance between the SHE assay and rodent bioassay is high. The mode of action has not been elucidated and therefore should be assumed relevant for humans
Mice (B6C3F1)	**Harderian gland** Adenoma or carcinoma.	Yes Tumours are observed in Harderian gland, lungs and liver	Yes	/	Both	No	Inhalation	No similar tissue is found in humans. The tissue is known to be sensitive to genotoxic compound but nitromethane was not found to be genotoxic.
	Lungs Alveolar / bronchiolar adenoma or carcinoma		Yes	No	Both	No	Inhalation	Non-genotoxic but a positive result was obtained in the SHE transformation assay

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	Liver Hepatocellular adenoma or carcinoma High background incidence		Yes	Yes	Only in females	No	Inhalation	The concordance between the SHE assay and rodent bioassay is high. The mode of action has not been elucidated and therefore should be assumed relevant for humans
NITROETHANE								
Rat (Long-Evans)	Pituitary adenoma Increase similar in controls No data on background incidence	No	No	/	Both	/	Inhalation	/

10.9.2 Comparison with the CLP criteria

CLP criteria cat. 1	CLP criteria cat. 2
Known or presumed human carcinogens A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as: Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: – human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or – animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with	Suspected human carcinogens The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited(1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

There is no information regarding carcinogenicity in humans. Therefore, Category 1A is not applicable.

To classify the substance on basis of carcinogenicity data in experimental animals, the following criteria are to be taken into account:

Classification in Category 1B: "*a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites*."

Classification in Category 2: "*the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the*

agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs."

Only one study performed with 1-nitropropane, not following any guideline, is reported in detail and showed a non-significant increased incidence of tumours (benign and malign) in rats (Griffin *et al., 1982)*, but in both exposed and control groups. Two other studies were poorly reported and the only available data mentioned that no increase was seen in the development of tumours in exposed animals, in comparison with the controls. Based on the available information on 1-nitropropane, the carcinogenic potential cannot be assessed properly.

One study, deviating from the OECD TG 453 (Anonymous 35, 1986), was available with nitroethane. In this study, only two doses were tested and no systemic effects were reported at the highest dose (200 ppm nitroethane).

The classification proposal for carcinogenicity of nitroethane and nitropropane is fully based on read-across from nitromethane because the available studies on nitroethane and 1-nitropropane are uninformative due to too low dosing and too low animal number. Thus, the key studies for the assessment of carcinogenicity are the 2-year studies in mice and rats on nitromethane (NTP, 1997).

Based on the fact that nitromethane induced an increased incidence of mammary tumours in female rats (statistically significant in carcinoma at the highest dose and in combination of benign and malignant tumours at the two highest doses which was also dose-dependent) (NTP, 1997), classification in category 1B or 2 has to be considered. The absence of overt toxicity at top dose and the earlier onset of these tumours in treated groups, in comparison with the control group, increases the concern as mammary gland tumours are usually observed at the end of life in rodents (NTP, 1997).

In a second independent study in rats (Anonymous 34, 1990), no increase in treatment-related tumours was induced but a reason could be that the doses used in this study were not high enough. The susceptibility of the two different strains to chemical carcinogenesis in the mammary gland was quoted similar (Wood *et al.*, 2002).

Overall, tumours in the mammary glands were statistically significantly increased in a dose-dependant manner in rats without confounding systemic toxicity and occurring earlier than in control animals (NTP, 1997). A dose-dependant increase in the severity of the lesions was also noted as statistically significant number of carcinomas were observed at the highest dose. These findings are therefore seen as treatment-related and are also supported by a slight increase in benign mammary gland tumours in female rats in a second study, although concluded less reliable due to some limitations in the study (dosing-strategy and absence of HCD amongst others). Finally, mammary tumour gland are considered relevant to human. Therefore, the observations of mammary gland tumours in female rat are concluded relevant for classification, in category 1B.

A second species (mice) was tested and tumours were observed in different tissues. Similar survival rates and comparable body weights between the treated and control groups suggest that the maximum tolerated dose was not reached in mice; while the top dose might have been too low, we can however conclude that the occurrence of neoplasms is unlikely to be caused by a general toxicity.

Indeed in mice malignant tumours such as alveolar/bronchiolar carcinoma were also observed in lungs of both sexes and this effect was dose-dependent. These tumours are consistent with the route of exposure. As HCD show that these tumours are not common in this strain of mice, there is a strong indication that these tumours are treatment-related. The DS notes also the relevance of these tumours to humans, which therefore warrants a classification, in category 1B.

An increased incidence of benign tumours of the liver was also observed in female mice and this increased incidence was confirmed when benign tumours were combined with malignant tumours. However, the strain used is known to spontaneously develop this type of tumours and the incidence of malignant tumours in all exposed mice was within the historical ranges. These tumours were not increased in male.

Finally, a significant dose-dependant increase of malignant tumours of Harderian glands was observed in male and female mice but this tissue has no equivalent in humans. The observation of Harderian glands

tumours in rodents is seen as an indication of the carcinogenic potential of the test-substance in the whole weigh-of-evidence analysis, especially when reported in association with other tumours (multi-site response). However, this tumour-type as such is considered not relevant to human.

The NTP paper (NTP, 1997) concludes "*Under the conditions of these 2-year inhalation studies, there was no evidence of carcinogenic activity of nitromethane in male F344/N rats exposed to 94, 188 or 375 ppm. There was clear evidence of carcinogenic activity of nitromethane in female F344/N rats based on increased incidences of mammary gland fibroadenomas and carcinomas. There was clear evidence of carcinogenic activity of nitromethane in male B6C3F1 mice based on increased incidences of harderian gland adenomas and carcinomas. There was clear evidence of carcinogenic activity in female B6C3F1 mice, based on increased incidences of liver neoplasms (primarily adenomas) and harderian gland adenomas and carcinomas. Increased incidences of alveolar/bronchiolar adenomas and carcinomas in male and female mice exposed to nitromethane were also considered to be related to chemical administration*"

The mode of action for the observed tumours is not identified. Nitromethane was not found genotoxic but a positive result was observed in a cell transformation assay. However, there are also non-genotoxic MoAs for carcinogenicity. There is no evidence showing or suggesting that the MoA(s) for the carcinogenic responses are not relevant to humans. Inflammation of the nasal tissue was reported in mice and is taken into account as a possible mode of action. It should be noted that inflammation is also a mode of action very relevant to humans.

IARC classified nitromethane for carcinogenicity in category 2B "possibly carcinogenic to humans". Furthermore, the DS notes as supporting evidence that the metabolism of nitromethane leads to the formation of formaldehyde which has a harmonised classification as Carc. 1B, H350 (https://echa.europa.eu/fr/information-on-chemicals/cl-inventory-database/-/discli/details/55163).

Nitromethane showed carcinogenic effects in two species (benign and malignat tumours were observed in mammary gland in rats and in liver and lungs in mice) in the absence of excessive toxicity and at doses relatively low. Based on the available dataset, the substance was not found to be genotoxic, however non-genotoxic mode(s) of action are relevant and should not be excluded. About the lungs tumours, olfactory epithelium degeneration was reported at a very high incidence, starting from the lowest dose (188 ppm) in mice. Local irritation, a relevant mode of action that could explain these severe effects and potentially the lungs tumours, is not mentioned in the study.

Therefore, classification as Carc. 1B, H350 (may cause cancer) is proposed. As no studies were performed using oral or dermal routes, a carcinogenic effect via these routes cannot be excluded and no specific route of exposure related to the classification is proposed.

10.9.3 Conclusion on classification and labelling for carcinogenicity

A classification **Carc. 1B, H350 (May cause cancer)** is proposed.

The route of exposure is not specified as it is not proven that no other routes of exposure cause the hazard.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 59: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
1-NITROPROPANE			
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Rat (SD) (Crl: CD(SD) IGSBR) 12/sex/dose OECD TG 422 GLP Reliability 1 (according to the registration dossier)	**1-nitropropane** Purity: 99.69 % Inhalation (vapours) Doses: 0, 25, 50 and 100 ppm (corresp. to approx. 0, 0.092, 0.184 and 0.369 mg/L) Actual conc. in chamber: 0, 24, 48 and 96 ppm Duration of exposure: 14 d of premating period, during mating for both sexes and until gestation day 19 for females	**Parental** Mortality: none Clinical signs: no effects observed BW: in males only: a trend to decrease was noted and was sign. lower at the highest dose at D7 of the premating period Organ weight: in males at highest dose: sign. lower FBW and sign. higher relative brain and relative testes weights **Sexual function and fertility** Reproductive performance: 2 females failed to become pregnant at the mid and high dose levels ***Developmental effects (assessed in sections 10.10.4-10.10.6)*** *Litter size: lower at the highest dose (not sign. however outside the range of HCD)* *Pup BW: sign. higher at 100 ppm in both sexes at lactation day 1 and 4 (within HCD range)*	Anonymous 37, 2003
NITROMETHANE			
13-week repeated dose inhalation toxicity study Rat (Fischer 344) 10/sex/dose No guideline	**Nitromethane** Purity: >98 % Doses: 0, 94, 188, 375, 750 or 1500 ppm (approx. equivalent to 0, 0.235, 0.47, 0.94, 1.87 and 3.74 mg/L, resp.)	Mortality: / BW: Sign. decrease in BW and BWG in males exposed to 1500 ppm Clinical signs: hindlimbs paralysis in all animals at 1500 ppm starting on day 21 and in some animals at 750 ppm starting from day 63 Hematology: dose-dependent microcytic responsive anemia	NTP, 1997

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
GLP-compliant Reliability 3 (according to the registration dossier, however report available to the DS and well documented)	Duration: 6h12min/d, 5 d/w, for 13 w	Organ weights: no changes **Sexual function and fertility:** Reproductive data: no significant change in the estrous cycle length significant decrease in sperm motility at 750 and 1500 ppm	
13-week repeated dose inhalation toxicity study Mice (B6C3F1) 10/sex/dose No guideline GLP-compliant Reliability 3 (according to the registration dossier, however report available to the DS and well documented)	**Nitromethane** Purity: >98 % Doses: 0, 94, 188, 375, 750 or 1500 ppm (approx. equivalent to 0, 0.235, 0.47, 0.94, 1.87 and 3.74 mg/L, resp.) Duration: 6h12 min/d, 5 d/w, for 13 w	Mortality: / BW: similar in all dose groups (except a slight increase at 375 ppm in females) Clinical signs: no data Organ weights: no effects **Sexual function and fertility:** Reproductive data: dose-dependent decrease in the sperm motility starting from 375 ppm. dose-related increase in the oestrous cycle length starting from 375 ppm.	NTP, 1997
NITROETHANE			
13-week repeated dose inhalation toxicity study Mouse (B6C3F1) 15/sex/dose Similar to OECD TG 413 Mainly GLP Reliability 2 (according to the registration dossier)	**Nitroethane** Purity: > 97 % Inhalation 6 h/d, 5 d/wk, 13 w Doses: 0, 100, 350, 1000 ppm (equivalent to 0, 0.3, 1.0, 3.0 mg/L, resp.)	**Parental toxicity:** No effect on BW, food consumption, clinical signs At 1000 ppm: Effects seen in the salivary glands, liver, and olfactory nasal epithelium At 350 ppm: Effects seen in liver, salivary glands and nasal turbinates and MetHb levels were affected At 100 ppm: Minimal changes reported (only in nasal turbinates and transiently in salivary gland epithelium) **Sexual function and fertility:** Sperm parameters not evaluated At 1000 ppm: Effects seen in the testes as significant increase of relative testicular weight and hyperplasia and multinucleated spermatids, effects in epididymes: at interim sacrifice slight focal unilateral decreased spermatogenesis in tubules (1/4 males),	Anonymous 26, 1982

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		slight focal unilateral interstitial hyperplasia in testis (1/4) and slight focal mononuclear aggregates in epididymis (1/4); at terminal kill very slight multifocal bilateral multinucleated spermatids (1/5), slight multifoc. bilat. multinucleated spermatids (1/5) and very slight multifoc. bilat. multinucl. spermatids in tubules (1/5) In females at terminal kill: primary benign teratoma in ovary (1/5), very slight focal muscularis acute inflam. in cervix (1/5) At 350 ppm: In testis, significant increase of relative testicular weight	
13-week repeated dose inhalation toxicity study Rat (F344) 15/sex/dose Similar to OECD TG 413 Mainly GLP Reliability 2 (according to the registration dossier)	**Nitroethane** Purity: >97 % Inhalation 6 h/d, 5 d/wk, 13 w Doses: 0, 100, 350, 1000 ppm (equivalent to 0, 0.3, 1.0, 3.0 mg/L, resp)	**Parental toxicity:** Statistically significantly decreased body weight in the 350 ppm (D49 for males and D61 for females) and 1000 ppm exposure groups (D44 in males and D61 for females) Cyanotic color of the skin (visible at 350 ppm after 9 w of exposure and in 1000 ppm after 4 exposure), dull and dark red eyes (visible at 350 ppm after 4 w of exposure and in 1000 ppm after the first exposure only) in both sex, unkept appearance in females No neoplastic lesions found at necropsy Effects on several absolute and/or relative organ weights. **Sexual function and fertility:** Relative testes weights were increased in a statistically significant way, in the 350 and 1000 ppm groups, in comparison with the controls.	Anonymous 26, 1982
Disregarded study Teratology study in mice Reliability 4 (according to the registration dossier)	/	Co-exposure to 8.9 ± 2.0 ppm diethylhydroxylamine and 14.3 ± 2.0 ppm nitroethane from GD 6 to GD 17 for 8.25 ± 2.25 h/d, 5 d/w. furthermore, continuous exposure to diethylamine hydrogen sulfite 24/7 also occured.	Beliles *et al.,* 1978
Disregarded study 3-generation toxicity study Reliability 4 (according to the registration dossier)	/	Co-exposure to 7.8 ± 1.2 ppm diethylhydroxylamine and 11.5 ± 2.9 ppm nitroethane for 8.25 ± 2.25 h/d, 5 d/w. Furthermore, continuous exposure to diethylamine hydrogen sulfite 24/7 also occured.	Heicklen *et al.,* 1979

No human data or other relevant information available.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Data on 1-Nitropropane

In a combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous 37, 2003), groups of 12 male and 12 female SD rats were exposed by inhalation (vapours) to 1-nitropropane (purity: 99.69 %) at a concentration of 0, 25, 50 or 100 ppm. Females were exposed 14 d prior to mating, during mating (2 w) and until the gestation day 19, whereas males were exposed 14 d prior to mating and during mating (for a minimum exposure of 28 d). Each female was placed with one male exposed to the same dose level.

All animals survived during the exposure period and did not exhibit any treatment-related clinical signs. A trend to lower body weight value was observed in males of the highest dose and the difference was significant at the day 7 of the premating period (see Table 60). These change were not observed in females (see Table 61).

Table 60: Body weight data in males (in g)

Dose level (in ppm)	0	25	50	100
D 1	288.8	287.6	290.0	282.8
D 7	317.0	315.0	319.1	295.0*
D 14	344.7	344.4	348.6	321.1
D 28	390.6	393.5	395.6	368.8

Table 61: Body weight data in females (in g)

Dose level (in ppm)		0	25	50	100
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

Reproductive performances were examined. No treatment-related effects on time to mating and gestation length were noted. However, 2 females failed to be pregnant at the mid and high dose levels (fertility index: 100, 100, 83.3 and 83.3 % respectively at 0, 25, 50 and 100 ppm, HCD (between 2000 and 2004: 83.3 and 100.0 %, for SD rats (Crl: CD(SD) IGSBR) of the same laboratory). It cannot be stated if the reduced fertility index can be attributed to male, female or unspecific causes. Plus, the reduction is still comprised within the historical control data range. However, the percentage of post-implantation loss was increased at 25 and 100 ppm with 5.43, 7.98, 3.97 and 7.06 % respectively at 0, 25, 50 and 100 ppm (HCD not available). No data is provided on sperm motility and morphology.

At necropsy, organ weight was examined. Males exposed to 100 ppm showed a significantly reduced final body weight value (354.1, 358.8, 357.3 and 328.7* g respectively at 0, 25, 50 and 100 ppm) as well as a significantly higher relative brain weight (0.562, 0.567, 0.572 and 0.622* g/100g respectively at 0, 25, 50 and 100 ppm) and relative testes weight (0.867, 0.902, 0.846 and 0.965* g/100g respectively at 0, 25, 50 and

100 ppm). Organ weights in females were not significantly changed. Histopathology examination revealed effects in females nasal tissue (such as multifocal degeneration of the olfactory epithelium, sometimes with signs of inflammation) (see Table 63).

Table 62: Organ weight data (in g and g/100g)

		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

Table 63: Incidence of nasal tissue degeneration

		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 olf. epith. (multifocal)	Very slight	0	0	0	1	0	0	0	5
	Slight	0	0	0	1	0	0	0	2
Degeneration of the olf. epith. with inflammation	Very	0	0	0	0	0	0	2	0

(focal)	slight								
Degeneration of the olf. epith. with inflammation (multifocal)	Slight	0	0	0	0	0	0	0	2
Chronic inflammation of the epith. (squamous cell) (focal)	Very slight	0	0	0	0	2	1	0	0
	Slight	0	0	0	0	0	0	0	1
Chronic inflammation of the epith. (squamous cell) (multifocal)	Very slight	0	0	0	0	1	1	1	2
	Slight	0	0	0	1	0	0	2	1

Litter examination revealed a slight decrease in mean litter size at the highest dose level (14.0, 14.3, 15.1 and 11.9 at birth respectively at 0, 25, 50 and 100 ppm; HCD 13.3 – 15.6). No more information that could explain this reduction was available in the full study report (e.g. on possible resorption or else).

Data on Nitromethane

In a 13-week repeated dose inhalation toxicity study in rats (NTP, 1997), 10 male and 10 female Fischer 344 rats were exposed to vapours of nitromethane (purity > 98 %) at doses of 0, 94, 188, 375, 750 or 1500 ppm (approx. equivalent to 0, 0.235, 0.47, 0.94, 1.87 and 3.74 mg/L, resp.) for 13 weeks. No mortality occurred during the study. BW and BWG were statistically significantly lower as compared to controls at study termination in males exposed to the highest dose (see Table 64). Hindlimbs paralysis was reported 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 from D63. Hematology findings showed a dose-dependent microcytic responsive anemia (with decreased Hg 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). No modifications were reported in organ weights.

Table 64: BW and BWG (in g)

Exposure level (ppm)		0	94	188	375	750	1500
♂	N	10	10	10	10	10	10
	BW at start	107 ± 3	105 ± 2	113 ± 2	109 ± 3	106 ± 2	109 ± 2
	FBW	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**
♀	N	10	10	10	10	10	10
	BW at start	95 ± 1	96 ± 2	97 ± 2	95 ± 2	96 ± 2	94 ± 2
	FBW	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

Concerning reproductive effects, a significant and dose-related decrease in sperm motility in males exposed to 750 or 1500 ppm was noted, in comparison with the control group. Furthermore, in the 1500 ppm group, a statistically significant decrease in testis, epididymis and cauda weights was reported. In males exposed to 1500 ppm, associated systemic toxicity was reported (significant decreased BW and BWG) and might have caused secondary effects. However, the dose-relationship and the fact that significant effects on sperm motility were seen at doses without any associated systemic toxicity suggest that the decrease in the sperm motility is treatment-related. Sperm morphology was not assessed.

No effects were observed in females' reproductive system or in estrous cycle. Reproductive organs tissues were not affected in either sex.

Table 65: Reproductive data

Exposure level (ppm)		0	375	750	1500
Males					
	N	10	10	10	10
Sperm parameters	Motility	94.57 ± 1.30	92.16 ± 1.90	87.11 ± 1.88**	76.43 ± 2.78**
	Count	64.33 ± 3.89	62.75 ± 3.63	62.68 ± 3.02	68.95 ±3.14
Weights (g)[a]	FBW 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					
	N	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

Sperm count: mean/10^{-4} mL suspension; L.= left; [a]= absolute

In a 13-week repeated dose inhalation toxicity study in mice (NTP, 1997), B6C3F1 mice (10/sex/dose) were exposed to vapours of nitromethane (purity > 98 %) at doses of either 0, 94, 188, 375, 750 or 1500 ppm (approximatively equivalent to 0, 0.235, 0.47, 0.94, 1.87 and 3.74 mg/L, respectively). No death occurred during the study. BW and BWG were similar in all dose groups. Organ weights were not affected in males. In females, heart weight (relative) was statistically significantly decreased at 375 ppm, in comparison with the controls, but not at lower or higher dose.

Table 66: Organ weights (in g or g/100 g)

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**

No effects were seen on cauda, epididymis or testis weights, or on sperm count. However, in males, adverse effect on the fertility was noted as the sperm motility was statistically significantly decreased at 375, 750 and 1500 ppm, in comparison with the control group. In females, the estrous cycle length was dose-dependently and significantly increased starting from 375 ppm, in comparison with the controls (4.00, 4.33*, 4.50* and 4.71** days in control, low, mid and high dose groups, respectively; no HCD available). No correlation between estrous cycle length and dams body weight could be highlighted. An oestrous cycle length increase is usually considered as an adverse effect related to normal oestrus cycle perturbation when it is associated with other effects such as hormonal dysfunction or any perturbation of the reproductive parameters. In contrast, the observations of oestrus cycle length impairment associated with decreased body weight can be seen as a secondary effect to systemic toxicity and therefore not relevant for reproduction toxicity classification. Here, in the absence of effects in females body weights between control and test-animals, the increased oestrus cycle length does not seem to be related to unspecific toxicity. On the other hand, it seems difficult to interprete the adversity of the observed increased oestrus cycle length in females based on the available dataset without further investigation. The DS however highlights that this effect seems to be treatment-related as it is clearly dose-dependent and statistically significant at all doses.

Table 67: 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**[c]

Table 68: 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

Data on Nitroethane

In a 13-week repeated dose inhalation toxicity study (Anonymous 26, 1982), rats were exposed to 0, 100, 350 and 1000 ppm corresponding to 0, 0.3, 1.0, 3.0 mg/L, respectively, for 6 h/d, 5 d/w for a total of 64-65 exposures (over a 92-d period) with an interim sacrifice of rats after 20-21 exposures (over a 30-d period). (See chapter 10.12 for detailed data)

No death occurred during the experiment. When exposed to the high dose level, a decreased in rats BW gain (see Table 102) 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 methemoglobin levels, spleen, nasal epithelium and salivary glands. The changes were minimal at 100 ppm in the methemoglobin level, spleen and salivary glands. Growth retardation was reported in the 1000 and 350 ppm in female and male rats. 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.

Two clinical findings, cyanosis and red eyes, were consistent with the grossly observable treatment-induced methemaglobinemia (Table 103).

- Dull, dark red eyes were very pronounced in the 1000 ppm group (appeared after the first exposure and thereafter), while ot was not very distinctive in the 350 ppm group (appeared after 4 weeks of exposure).

- Grayish or bluish colored skin of the extremities (cyanosis) was reported in the 350 ppm group after 9 weeks of exposure and in the 1000 ppm group after 4 exposure and thereafter. Effects disappeared within 19 hours after exposure, in both treatment groups.

- 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 occured in this laboratory and were not judged to be treatment-related.

Prior to interim kill (20th exposure day, D29 of the experiment), methemoglobin was dosed in blood (see Table 103), 15 hours after the last exposure (Part A of Table 103). 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 methemoglobinemia 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 Table 103).

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 Table 103).

At terminal kill, a time-sequenced analyse (Part D of Table 103) was performed less than 30 min after exposure, 4 and 19h 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.

Prior to the interim kill (30 days), statistically significant lowered hemoglobin values in male rats and statistically significant increases of the WBC counts were seen in the 1000 ppm group. At 350 and 1000 ppm, increased levels of reticulocytes and Heinz bodies were noted.

Prior to the terminal kill (92 days), a statistically significant increased PCV and a decreased RBC count was noted in females as well as statistically significant lowered hemoglobin values in male rats, at 1000 ppm. At 350 and 1000 ppm, increased levels of reticulocytes and Heinz bodies were noted. (Table 104).

Reproductive tissues were examined and an increase of relative testis weight was detected in the highest dose at interim and final sacrifice.

Table 69: Testes weight at interim kill (in g)

Dose level (in ppm)	0	100	350	1000
Body weight	229.8 +/- 13.5	219.6 +/- 9.3	216.6 +/- 8.3	203.8* +/- 9.3

Testes	Abs	2.92 +/- 0.17	2.75 +/- 0.06	2.80 +/- 0.08	2.81+/- 0.06
	Rel	1.27 +/- 0.04	1.25 +/- 0.06	1.29 +/- 0.03	1.38* +/- 0.05

Table 70: Testes weight at final kill (in g)

Dose level (in ppm)		0	100	350	1000
Body weight		229.0 +/- 13.2	295.1 +/- 17.8	289.7 +/- 10.0	264.2* +/- 15.6
Testes	Abs	2.94 +/- 0.24	3.15* +/- 0.18	2.99 +/- 0.13	2.98 +/- 0.14
	Rel	0.99 +/- 0.09	1.07 +/- 0.12	1.03 +/- 0.03	1.13* +/- 0.03

Table 71: Histopathological observations (at terminal kill)

Dose level (ppm)	0	100	350	1000
N examined	5	5	5	5
Males				
N testes tissues assessed	5	5	5	5
Normal testes	5	4	5	5
Diminished spermatogenesis	0	1 S.	0	0
MetHb (% ± St. Dev)	0.4 ± 0.4	2.4 ± 0.5	12.9* ± 5.4	50.7* ± 5.4
Females				
N uterus examined	5	5	5	5
Normal cycle changes	0	0	1	0
N Mammary gland examined	4	3	5	5
Slight hyperplasia in acini	0	1	0	0
Slight hyperplasia in ducts	0	0	1	1
MetHb (% ± St. Dev.)	0.5 ± 0.3	5.3 ± 1.7	30.7* ± 3.9	61.8* ± 6.0

S. = slight, V.S.= very slight, b.= bilateral, m.= multifocal

In a 13-week repeated dose inhalation toxicity study (Anonymous 26, 1982), mice were exposed to 0, 100, 350 and 1000 ppm 6 h/d, 5 d/w. Decreased BW was noted (see chapter 10.12 for detailed data). Cyanotic color of the skin, dull and dark red eyes were reported in both sex. Unkept appearance was seen in females.

Reproductive tissues were examined. At 1000 ppm, effects were seen in the testes (multinucleated spermatids, significant increase of relative weight), the salivary glands, the liver, and nasal epithelium. At 350 ppm, the significant increase of testis weight was already visible. Effects were also seen in liver, salivary glands and nasal turbinates and MetHb levels were also affected. Minimal modifications were reported in mice exposed to 100 ppm and changes were observed only in nasal turbinates and transiently in salivary gland epithelium.

Table 72: Testes weight at terminal kill

Dose level (in ppm)	0	100	350	1000

Body weight		34.3 +/- 2.0	33.6 +/- 2.5	32.4 +/- 2.6	32.4 +/- 2.5
Testes	Abs	0.22 +/- 0.02	0.22 +/- 0.02	0.23 +/- 0.02	0.23 +/- 0.02
	Rel	0.64 +/- 0.06	0.65 +/- 0.05	0.70* +/- 0.05	0.72* +/- 0.03

Table 73: Methemoglobin levels (%±St. Dev.) after last exposure

Dose level (ppm)	0	100	350	1000
N examined	5	5	5	5
Males				
Methb	0.8 ± 0.3	1.2 ± 0.4	6.6* ± 4.3	36.4* ± 3.0
Females				
Methb	1.2 ± 0.7	0.9 ± 0.7	5.8* ± 1.8	20.8* ± 2.0

Table 74: Histopathological observations (at terminal kill)

Dose level (ppm)	0	100	350	1000
Males				
N tissues assessed	5	0	0	5
No Lesions on testes recognized	4	/	/	2
Testes degeneration (focal)	1 S.	/	/	0
Multinucleated spermatids, b., m.	0	/	/	1 V.S. 1 S.
Multinucleated spermatids tubules, b., m.	0	/	/	1 V.S.
No lesions on epidydimis recognized	5	/	/	5
No lesions on seminal vesicle recognized	5	/	/	5
No lesions on prostate recognized	5	/	/	5
No lesions on coagulated gland recognized (Nb with no lesion/nb examined)	3/3	/	/	2/2
Females				
N tissue examined	5	0	0	5
No lesions on ovary recognized	5	/	/	4
Benign teratoma, no meta., primary	0	/	/	1
No lesions on oviduct recognized	5	/	/	5
No lesions on uterus recognized	5	/	/	5
No lesions on cervix recognized (/nb examined)	4/4	/	/	4/5
Cervix: Acute inflammation muscularis, focal	0	/	/	1 V.S.

S. = slight, V.S.= very slight, b.= bilateral, m.= multifocal

10.10.3 Comparison with the CLP criteria

CLP criteria cat. 1	CLP criteria cat. 2
"Known or presumed human reproductive toxicant Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (category 1A) or from animal data (Category 1B)."	"Suspected human reproductive toxicant Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be the more appropriate classification. Such effect shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be secondary non-specific consequence of the other toxic effects."

Since no human studies are available for effects fertility, classification in Repr. 1A is not appropriate.

- Sperm parameters:

Sperm was examined in two studies performed with nitromethane. As observed in Table 75, these two studies revealed that sperm was affected by treatment. In the 13-week repeated dose inhalation toxicity study in rat (NTP, 1997), a significant and dose-dependent decrease in sperm motility was evidenced with 94.57, 92.16, 87.11** and 76.43** % at 0, 375, 750 and 1500 ppm. This was also reported in mice. Indeed, in the 13-week repeated dose inhalation toxicity study in mice (NTP, 1997), a significant decrease in sperm motility was observed with 93.5, 85.09**, 86.47** and 82.42** % at 0, 375, 750 and 1500 ppm, respectively. The decrease in sperm motility observed is considered treatment-related based on a dose-dependance and a statistical significance at mid and high dose in two different species. In addition, the absence of body weight loss in mid-dose animals indicates that the decreased sperm motility cannot be linked to unspecific systemic toxicity. It should be noted that these 13-week repeated dose inhalation toxicity studies are not reproductive toxicity studies, the study design therefore implies that the reproductive effects are moderate and cannot be associated with a potential decrease of the reproductive function (such as litter size or the number of pregnant dams). However, the effects were reported at dose level which also showed concentration-dependent microcytic responsive anemia. As reported in Reyes *et al.* study (2012), hypoxia can lead to adverse effects on spermatogenesis. Nevertheless, the article mentions that "*A reduced sperm count can be related to the increase in germ cell apoptosis promoted by this hypoxic condition. The same results were observed in male rhesus monkeys. Morphological studies have revealed that chronic hypoxia causes degeneration of the germinal epithelium, folding of the basement membrane, degeneration and detachment of germ cells, changes in lipid droplets in Sertoli cells, and an increase in lipoperoxidation. Other local changes in the testicles have also been observed, including an increase in vascularization, an increase in testicular temperature, a decrease in testicular mass, and an increase in interstitial space*". Other effects which were not observed in the available studies. The CLP guidance noted that "*Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary nonspecific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate*".

Sperm parameters were not examined in the available studies performed with 1-nitropropane or with nitroethane. However, in the combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonyous 37, 2003), two females exposed to 50 and 100 ppm failed to be pregnant, resulting in a fertility index of 100.0, 100.0, 83.3 and 83.3 %, resp. at 0, 25, 50 and 100 ppm. The reduction wass just within the range of the HCD (83.3 to 100.0 %). However, it cannot be stated if the decrease could be attributed to male or female causes.

- Male reproductive organ:

As observed in Table 75, male reproductive organ exhibited variation in different studies. Some of them were significant.

Table 75: Male fertility parameters

	Sperm parameters	**Reproductive organ weight**
Nitromethane		
13-week repeated dose inhalation toxicity study in (Fischer 344) rat (NTP, 1997)	Motility: 94.57, 92.16, 87.11 and 76.43 %, resp. at 0, 375, 750 and 1500 ppm Sperm count: 64.33, 62.75, 62.68 and 68.95 10^{-4} mL suspension, resp. at 0, 375, 750 and 1500 ppm	L. cauda: 0.207, 0.210, 0.204 and 0.177**g, resp. at 0, 375, 750 and 1500 ppm L. epididymis: 0.467,0.468, 0.444 and 0.412** g, resp. at 0, 375, 750 and 1500 ppm L. testis: 1.39, 1.36, 1.34 and 1.29** g, resp. at 0, 375, 750 and 1500 ppm
13-week repeated dose inhalation toxicity study in (B6C3F1) mice (NTP, 1997)	Motility: 93.5, 85.09**, 86.47**, 82.42** %, resp. at 0, 375, 750 and 1500 ppm	Unaffected
Nitroethane		
13-week repeated dose inhalation toxicity study in mice (Anonymous 26, 1982)	Not examined	Testes: 0.22, 0.22, 0.23 and 0.23 g, resp. at 0, 100, 350 and 1000 ppm (rela weight: 0.64, 0.65, 0.70* and 0.72* %, resp. at 0, 100, 350 and 1000 ppm)
1-Nitropropane		
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonyous 37, 2003)	Not examined	Epididymide: 1.024, 1.070, 1.038 and 1.054 g resp. at 0, 25, 50 and 100 ppm (rela weight: 0.290, 0.299, 0.291 and 0.322 %) Testes: 3.066, 3.230, 3.015 and 3.162 g resp. at 0, 25, 50 and 100 ppm (rela weight: 0.867, 0.902, 0.846 and 0.965* %)

- Female reproductive organ:

In the 13-week repeated dose inhalation toxicity study in rat (NTP, 1997) performed with nitromethane, oestrous cycle length was not significantly affected. However in the same study performed in mice (NTP, 1997), it was signicantly and dose-related increased at the 3 tested doses (4.00, 4.33*, 4.50* and 4.71**, resp. at 0, 375, 750 and 1500 ppm). No studies performed with nitroethane and 1-nitropropane examined the

oestrous cycle length. As mentioned before, in the Combined Repeated Dose Toxicity with the Reproduction/Developmental Toxicity Screening Test (Anonyous 37, 2003), two females exposed to 50 and 100 ppm failed to be pregnant, resulting in a fertility index of 100.0, 100.0, 83.3 and 83.3 %, resp. at 0, 25, 50 and 100 ppm. The reduce was just within the range of the HCD (83.3 to 100.0 %). However, it cannot be stated if the decrease could be attributed to male or female causes.

Table 76: Female fertility parameters

	Estrous cycle	Fertility index	Gestation length
Nitromethane			
13-week repeated dose inhalation toxicity study in rat (NTP, 1997)	4.89, 4.75, 5.00 and 5.00 d, resp. at 0, 375, 750 and 1500 ppm	/	/
13-week repeated dose inhalation toxicity study in mice (NTP, 1997)	4.00, 4.33*, 4.50* and 4.71**, resp. at 0, 375, 750 and 1500 ppm	/	/
Nitroethane			
13-week repeated dose inhalation toxicity study (Anonymous 26, 1982)	Not examined	/	/
1-Nitropropane			
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous 37, 2003)	Not examined	Reduced at the 2 highest dose: 100, 100, 83.3 and 83.3 % resp at 0, 25, 50 and 100 ppm (HCD: 83.3 – 100 %) 2 F at the mid and high doses failed to be pregnant	21.3, 21.5, 21.4 and 21.8 d

Conclusion:

The DS concludes that there is some evidence on the adverse effects on sexual function and fertility and proposes a classification as **Repro. 2; H361f for adverse effects on sexual function and fertility.**

10.10.4 Adverse effects on development

Table 77: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
1-NITROPROPANE			
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Rat (SD) 12/sex/dose OECD TG 422 GLP Reliability 1 (according to the registration dossier)	**1-nitropropane** Purity: 99.69 % Inhalation (vapours) Doses: 0, 25, 50 and 100 ppm (corresp. to approx. 0, 0.092, 0.184 and 0.369 mg/L) Actual doses: 0, 24, 48 and 96 ppm Duration of exposure: 14 d of premating period, during mating for both sexes and until gestation day 19 for females	**Maternal/paternal effects** Mortality: / Clinical signs: no effects observed BW: a trend to decrease was noted in males and was sign. lower at the highest dose at D7 of the premating period Organ weight: in males: sign. lower FBW and sign. higher relative brain and relative testes weights **Developmental effects** Post-implantation loss: 5.43, 7.98, 3.97 and 7.06 % resp. at 0, 25, 50 and 100 ppm Litter size: lower at the highest dose (not sign. however outside the range of HCD) Pup BW: sign. higher at 100 ppm in both sexes at lactation day 1 and 4 (within HCD range)	Anonymous 37, 2003
NITROMETHANE			
Prenatal Developmental Toxicity Study Rat (Wistar) 24 females/group (2 females mated with 1 male) OECD TG 414 GLP Reliability 1 (according to the registration dossier) Deviations: identification of males via a subcutaneous transponder and not a mark on the tail, variation of the	Nitromethane Purity: > 99 % Inhalation (vapours) Doses: 0, 300, 600 and 1200 ppm (± 0, 0.75, 1.50 and 3 mg/L, resp.) Duration of exposure: 6 h/d, from GD 6 to 20	Actual conc. in chamber: 303, 601 and 1178 ppm (similar to 0.75, 1.50 and 2.99 mg/L, resp.) **Maternal toxicity:** Mortality: / Clinical sign: no abnormal change reported BW: sign. decreased at days 18 and 21 at 1200 ppm BWG: sign. decreased from D15 to D21 Organ weight: sign. decreased relative ovaries, relative liver,	Anonymous 36, 2017

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
relative humidity from 44.9 to 65 % and no use of the surplus animals for training purpose.		absolute and relative kidney weights at 1200 ppm Food consumption: stat. sign. decreased between days 6-9 and 18-21 at 1200 ppm Parental necropsy: no treatment-related macroscopic modification observed **Developmental effects:** Post-implantation loss: stat. sign. increase in the % of late resorptions and in % of post-implantation loss at 1200 ppm Number of foetuses: stat. sign. decrease in the mean number of foetuses per dam at 1200 ppm Gravid uterus weight: stat. sign. decreased gravid uterus weight at 1200 ppm Pup bw: at 1200 ppm stat. sign. decreased BW at birth, in both sexes Developmental abnormalities (including malformations): stat. sign. increase in the % of pale foetuses per litter, in the % of foetuses with variations per litter, in the % of malformed foetuses per litter, in the % of foetuses with skeletal variations/litter	
Disregarded study Reproductive toxicity study in rat Reliability 4 (according to the registration dossier)	/	Maze learning impaired in all treated groups with histidine diet groups more affected than the nitromethane condition.	Whitman *et al.*, 1977
NITROETHANE			
Disregarded study Teratology study in mice Reliability 4 (according to the registration dossier)	/	Co-exposure to 8.9 ± 2.0 ppm diethylhydroxylamine and 14.3 ± 2.0 ppm nitroethane from GD 6 to GD 17 for 8.25 ± 2.25 h/d, 5 d/w. furthermore, continuous exposure to diethylamine hydrogen sulfite 24/7 also occured.	Beliles *et al.,* 1978
Disregarded study 3-generation toxicity study	/	Co-exposure to 7.8 ± 1.2 ppm diethylhydroxylamine and 11.5 ± 2.9 ppm nitroethane for 8.25 ± 2.25 h/d, 5 d/w. Furthermore, continuous exposure to diethylamine hydrogen	Heicklen *et al.,* 1979

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Reliability 4 (according to the registration dossier)		sulfite 24/7 also occured.	

No human data or other relevant studies available.

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

Please also refer to Chapter 10.10.2

Data on 1-Nitropropane

In a combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous 37, 2003), groups of 12 male and 12 female SD rats were exposed by inhalation (vapours) to 1-nitropropane (purity: 99.69 %) at a concentration of either 0, 25, 50 or 100 ppm. Females were exposed 14 d prior to mating, during mating (2 w) and until the gestation day 19, whereas males were exposed 14 d prior to mating and during mating (for a minimum exposure of 28 d). Each female was placed with one male exposed to the same dose level.

As mentioned in chapter 10.10.2, all animals survived during the exposure period and did not exhibit clinical signs. Body weight and organ weight were unaffected in females, and histopathological examination revealed nasal tissue modifications (see chapter 10.10.2 for further information).

Concerning developmental effects, the percentage of post-implantation loss per litter was modified (5.43 ± 7.04, 7.98 ± 7.64, 3.97 ± 4.65 and 7.06 ± 10.71 % respectively at 0, 25, 50 and 100 ppm, no HCD available). Litter examination revealed a decrease in mean litter size at the highest dose level (mean ± St.Dev.: 14.0 ± 1.8, 14.3 ± 2.1, 15.1 ± 1.7 and 11.9 ± 4.3 live pups at birth respectively at 0, 25, 50 and 100 ppm; HCD 13.3 – 15.6; HCD 2000-2004, from the same laboratory, SD rats). Individual data showed that 1/12, 1/12, 0/10 and 3/10 dams had litter size inferior than 12 pups at 0, 25, 50 and 100 ppm, respectively.

Considering these observations, the DS is of the opinion that litter size reduction at the highest dose may be caused by the treatment. No mortality was reported during the study period, neitheir behavior, nor food consumption and nor body weight of the dams were impacted throught the study by treatment. Furthermore, the available individual data do not allow to determine the cause of the reduced litter size such as individual data on post-implantation loss which could have been compared to individual data on litter size to see if the reduction in the latter was due to post-implanatation loss or not. The DS also notes that an even greater percentage in post-implantation loss was observed at 25 ppm, however the mean litter size in the lowest dose group is still similar to the control and mid-dose groups.

The survival index and sex ratio were unaffected (see Table 78). However, at the highest dose, a significantly higher pup body weight was noted in both sexes at PND 1 and 4, but it was included within the HCD (see Table 79). Variations and malformations were not examined in the study as well as the physical landmarks.

Table 78: Developmental data

Dose level (in ppm)		0	25	50	100
Sex ratio (males/females)		46/54	51/49	48/52	51/49
Survival index	At birth	98.8 (168/170)	99.4 (171/172)	99.3 (151/152)	99.2 (119/120)
	At D 1	98.8 (166/168)	100 (171/171)	100 (151/151)	99.2 (118/119)
	At D 4	98.8 (166/168)	98.8 (169/171)	100 (151/151)	99.2 (118/119)

Table 79: Pup body weight data (in g)

	Males					Females				
Dose level (in ppm)	0	25	50	100	HCD	0	25	50	100	HCD
D 1	6.7	6.9	6.6	7.3*	7.0 – 7.4	6.3	6.5	6.2	6.9*	6.5 – 7.0
D 4	9.2	9.7	9.2	10.4*	9.6 – 10.7	8.8	9.2	8.6	9.7*	9.1 – 10.7

Data on Nitromethane

In a prenatal developmental toxicity study in rat (Anonymous 36, 2017), 24 pregnant females per dose groups were exposed to nitromethane at concentrations of either 0, 300, 600 or 1200 ppm (approximatively equivalent to 0, 0.75, 1.50 and 3 mg/L, respectively), 6 h/d, from GD 6 to 20. No mortality occurred in either dose group.

Body weights were statistically significantly decreased at days 18 and 21 in females exposed to the highest dose as compared to controls. This can be explained by a statistically significantly decreased gravid uterine weight in dams of the highest dose group (see Table 83).

No abnormal change was reported in clinical signs.

Table 80: BW at the start of the study in females and evolution during gestation (in g)

Dose (ppm)	0	300	600	1200
N	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 81: BW gain (g) in females, during gestation

Dose (ppm)	0	300	600	1200
N	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 was not significantly different between the dose groups, except between days 6-9 and 18-21, where the food consumption was statistically significantly lower in females exposed to 1200 ppm as compared to controls. The decreased food consumption in the highest dose group is consistent with the decreased BWG in females at the same time points and the reduced litter size.

Table 82: Food consumption (g) in females

Dose (ppm)	0	300	600	1200
N	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

No treatment-related macroscopic modifications were observed during dams necropsy. No data is available on hematology or serum chemistry analyses.

Organ weight findings reported statistically significantly decreased gravid uterus (due to significantly reduced litter size), relative ovaries, relative liver, absolute and relative kidney weights in females exposed to 1200 ppm.

Table 83: Organ weights (g) in females

Dose (ppm)	0	300	600	1200
Terminal BW (D21)	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 (abs) (g)	10.7228 ± 0.9706	11.3909 ± 0.8206	10.9018 ± 0.9298	11.3716 ± 1.0548
Liver (rel) (%)	3.2572 ± 0.2065	3.3789 ± 0.2048	3.3632 ± 0.3029	3.9670** ± 0.2843
Kidneys (abs) (g)	1.3716 ± 0.1276	1.4724* ± 0.1175	1.4840* ± 0.1179	1.6044** ± 1.1222
Kidneys (rel) (%)	0.4175 ± 0.0384	0.4366 ± 0.0276	0.4576 ± 0.0357	0.5623** ± 0.0631

Several developmental parameters were statistically significantly altered at the highest dose. A statistically significant increase in the percentage of late resorptions and of post-implantation loss were reported as well as a statistically significant decrease in the mean number of foetuses per dam at 1200 ppm. In the 1200 ppm group, the mean percentage of post-implantation loss was greatly increased to 53.8 %. The authors stated that it was partly caused by a complete litter loss in 5 out of 22 females. If these females are not included in calculations, the corrected post-implantation loss was 38 % for females having at least one live foetus in her litter.

Table 84: Reproductive parameters

Dose (ppm)	0	300	600	1200
Nb of dams examined	17	19	20	22
Mean nb corpora lutea/dam	14.1	14.2	12.9	13.6
Mean nb 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-implantation loss/dam	0.3	0.3	0.5	6.9**
% Post-implantation 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

Foetuses BW was significantly decreased at 1200 ppm, in males and females (Table 85). A significant increase in the percentages of pale foetuses per litter, of foetuses with variations per litter, of malformed foetuses per litter and of foetuses with skeletal variations/litter was observed, as reported in Table 86 and Table 87. Hematological parameters were not monitored in dams, nor in foetuses.

Table 85: Foetal body weights (g)

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

Subcutaneous edema, listed as external malformation, was seen on one foetus from the high dose group. Regarding variations, subcutaneous hemorrhages were reported on two foetuses, one in the control group and one in the high dose group. Furthermore, in the high dose group, a statistically significant increase in the number of pale foetuses (13/17 litters) was recorded. No effects were seen in the low and mid dose groups. No visceral malformation were observed in any dose group.

Table 86: Effects on foetuses (external malformations and variations)

Doses (ppm)	0	300	600	1200
N foetuses examined	202	227	223	126
N litters examined	17	19	20	17
Malformations				
N foetuses with Malformations (N 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
N External malformation (%/litter)	0 (0)	0 (0)	0 (0)	1 (0.05)
N foetuses with Subcutaneous edema (%/litter)	0 (0)	0 (0)	0 (0)	1 (0.05)
Variations				
N foetuses with variations (N 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**
Total N ext. variations (%/litter)	1 (0.5)	0 (0.0)	0 (0.0)	105 (76.52**)
N litters affected with ext. variations (% of affected litters)	1 (5.9)	0	0	13** (76.5)
N foetuses with subcutaneous haemorrhage (%/litter)	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.8)
N Pale foetuses (%/litter)	0 (0.0)	0 (0.0)	0 (0.0)	105 (76.5**)

Skeletal malformations examination revealed that 2.2, 0.0, 0.7 and 16.4 % of foetuses were affected per litter, with 11.8, 0, 5.0 and 29.4 % of the litters affected at 0, 300, 600 and 1200 ppm, respectively. It consisted mainly of one absent and one branched rib in the control group (same animal) and of split sternebra in the 1200 ppm group (8 cases out of 9 foetuses with skeletal malformations; on a total of 69 pups examined). The other skeletal malformation was a fused sternebra reported in one foetus at the highest dose. Skeletal variations affected 97.1, 99.1, 95.8 and 100 % of the examined foetuses, at 0, 300, 600 and 1200 ppm, respectively. Significant increase in the percentage of foetuses affected per litter was mostly seen only at the high dose. The table below shows some of the observed variations.

Table 87: Skeletal defects in foetuses

Doses (ppm)	0	300	600	1200
N foetuses examined	105	119	118	69
N litters examined	17	19	20	17
Malformations				
N foetuses with skel. malformations (N litters affected)	2 (2/17)	0 (0/19)	1 (1/20)	10 (5/17)
N foetuses with ribs malformed	1	0	0	0
N foetuses with sternebra malformed (%/litter)	0	0	0	9 (10.5**)
Variations				
N foetuses with variations (% per litter)	103 (97.1)	118 (99.1)	114 (95.8)	69 (100)
N 1-4 unossified digits (% per litter)	23 (21.0)	23 (20.1)	25 (20.5)	49 (65.6**)
N incomplete ossification pubis (%/litter)	0	0	0	6 (14.2*)
Wavy ribs	1 (1.0)	3 (2.6)	18 (14.7*)	34 (47.3**)
Incomplete ossification Metatarsals (hindlimbs)	26 (23.1)	20 (17.0)	44 (36.8)	55 (74.9**)

In a non-guideline study aiming to assess the learning ability impairment in pups potentially caused by high histidine exposure *in utero* (Whitman *et al.*, 1977), 4 groups of female albino rats received a special diet and/or ip injection for a week. Histidine levels in urine was examined at the end of the week of treatment. As all females showed elevated leveld of histidine in urine, 2 males per group were introduced until occurrence of impregnation. Exposure of the dams continued and levels of histidine were monitored qualitatively during the gestation. The groups were defined as follow:

1- Control group: control diet, fixed quantity per day, normal daily amount of histidine + ip injection of 0.5 ml of 0.9 % NaCl every 3 days

2- Histidine diet: daily fixed amount of high-histidine diet + ip injection of 0.5 ml of 0.9 % NaCl every 3 days

3- Nitromethane injected: daily fixed amount of control diet + ip injection of 0.5 ml of 1.5 M nitromethane in 0.9 % NaCl, every 3 days

4- Histidine diet + nitromethane injected: daily fixed amount of high-histidine diet + ip nitromethane injection every 3 days, as described above

The fixed amount of diet was similar in all groups. Successful matings percentage, and litter size were equivalent in all groups and subsequent pups survival rates were relatively high in all groups (no more data). Dams behaviour towards their offspring was similar in all groups and therefore unaffected by the treatment. No significant difference in birth weight was observed, however, the BWG tended to be lower during the first month in groups exposed to high-histidine diet. When behavioural testing began, all animals from all groups had an average BW of 250 g. Animals were then randomly selected from the 16 litters, stayed with their mother until weaning then kept on a control diet *ad libitum* until they were 2-month old. *Ad libitum* feeding period was restrained to 1 hour per day for two weeks and when animals were 2 month ½ old, behavioural testing was started and consisted of maze box (design developed by Hebb and Williams in 1946 and described by Davenport *et al.*, 1970).

10 rats per group were selected, learned one maze per day and passed the test until they achieved a 4 out of 5 errorless trial. Analysis of the errors to the criterion developed by Hebb-Williams showed that the control and the nitromethane groups had results significantly different ($p < 0.05$). The control diet groups and high-histidine diet groups had significantly different results ($p < 0.05$), but the latter groups had not significantly different results compared to each other.

The percentage of trials with exactly similar pattern of errors (eg. As in a previous trial) was monitored and analysis of variance showed significant difference between the control and experimental groups ($p < 0.05$), but nitromethane group was not significantly different that the high-histidine diet groups. High-histine diet groups were not significantly different from each other as well.

In conclusion, maze learning was impaired in all treated groups with histidine diet groups more affected than the nitromethane condition. These results were expected if they are caused by a high histidinemia in pregnant dams and subsequent high-histidine levels exposure *in utero* of the offspring. Histidinemia in the nitromethane groups was not as high as in the high-histidine diet group. *In utero* exposure was sufficient to induce learning impairment in the offspring.

Data on Nitroethane

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10.10.6 Comparison with the CLP criteria

CLP criteria cat. 1	CLP criteria cat. 2
"Known or presumed human reproductive toxicant Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans.	"Suspected human reproductive toxicant Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the

The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (category 1A) or from animal data (Category 1B).” Category 1A: Known human reproductive toxicant The classification of a substance in this Category 1A is largely based on evidence from humans. Category 1B: Presumed human reproductive toxicant The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary nonspecific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.	quality of evidence less convincing, category 2 could be the more appropriate classification. Such effect shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be secondary non-specific consequence of the other toxic effects.”

Table 88: Summary of developmental data

	Post-implantation loss	Litter size	Survival index at D 4	Pups body weight	Malformation and variation
Nitromethane					
Prenatal developmental toxicity study (Anonymous 36, 2017)	Significantly higher 2.2, 2.1, 3.9 and 53.8** %	Significantly reduced 11.9, 11.9, 11.2 and 5.7**	/	Foetal bw: 4.96, 5.10, 4.98 and 3.93** g in males and 4.80, 4.91, 4.76 and 3.65** g in females	Significant increase incidence of pale foetus at the highest dose (76.5 %/litter) + Sternebra malformed, wavy ribs, incomplete ossification of metatarsal, incomplete ossification of pubis
Nitroethane					
No study available					
1-Nitropropane					

Combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonyous 37, 2003)	5.43, 7.98, 3.97 and 7.06 %	14.0, 14.3, 15.1 and 11.9 Reduced at the highest dose Not dose related Outside range HCD (13.3 – 15.6)	98.8, 98.8, 100 and 99.2 %	At D 1: 6.7, 6.9, 6.6 and 7.3* g in males and 6.3, 6.5, 6.2 and 6.9* g in females At D 4: 9.2, 9.7, 9.2 and 10.4* g in males and 8.8, 9.2, 8.6 and 9.7* g in females	Not reported

Since no human studies are available for effects on fetal development, classification in Repr. 1A is not appropriate.

In the combined repeated dose toxicity with reproductive/developmental screening toxicity study (Anonymous 37, 2003), the percentage of post-implantation loss showed variations but was not significantly affected (5.43, 7.98, 3.97 and 7.06 % respectively at 0, 25, 50 and 100 ppm; corresponding to approx. 0, 0.092, 0.184 and 0.369 mg/L). The mean litter size at birth was lower at the highest dose level (11.9 vs 14.0 in control group, this value was outside the HCD range: 13.3 – 15.6). Malformations and variations were not assessed in this study. These effects were observed at a very low dose (100 ppm 1-nitropropane corresponding to approximatively 0.369 mg/L).

In a prenatal developmental toxicity study, performed with nitromethane (Anonymous 36, 2017), developmental effects were described. A significant increase was reported in the percentages of late resorptions and post-implantation loss at the highest dose (with 2.2 and 53.8 % post-implantation loss at 0 and 1200 ppm, respectively). Furthermore, a significant decrease was noted in the mean number of foetuses per dam (11.9 and 5.7 at 0 and 1200 ppm, respectively) as well as in foetuses body weights (in average 4.8 and 4.96 g at 0 ppm; and 3.65 and 3.93 g at 1200 ppm, in males and females, respectively). Finally, a significant increase in the number of pale foetuses (0 and 76.5 % per litter, at 0 and 1200 ppm, respectively), in the number of foetuses with malformations 1.2 and 8.4 % foetuses with malformations, at 0 and 1200 ppm, respectively; the number of litters affected was 2 and 5 out of 17, at 0 and 1200 ppm, respectively) or variations (0.5 and 76.52 % at 0 and 1200 ppm, respectively) and with skeletal malformations (2.2 and 16.4 %, at 0 and 1200 ppm, respectively) were observed. Pale foetuses was an observation consistent with haematological effects seen on the rat after exposure to nitromethane (increased methemoglobinemia, anemia) in the 13-week repeated dose inhalation toxicity study (NTP, 1997; Lewis *et al*., 1977; refer also to chapter 10.12). All these developmental effects appeared at the highest dose only (1200 ppm, equivalent to 2.99 mg/L) in the absence of dose-relationship or severe maternal toxicity. Indeed, no mortality occurred in the dams during the study and no clinical signs are reported. BW, BWG and food consumption were significantly reduced. Food consumption was only significantly reduced during the periods GD 6-9 and GD 18-21, during the reste of the period, it was only slightly reduced. Regarding the reduce BW and BWG, these modifications were expected since the number of foetuses per dams was significantly decreased at the high dose, in comparison with the controls.

The classification proposal is based on the read-across with nitromethane as there is no prenatal developmental toxicity study performed on 1-nitropropane and nitroethane. In the available prenatal developmental toxicity study performed with nitromethane (Anonymous 36, 2017), clear evidence of effects on developmental parameters were observed considered not secondary to maternal toxicity which is in line with a classification in category 1B.

The DS is of the opinion that a classification as **Repr. Cat. 1B, H360D** is warranted.

10.10.7 Adverse effects on or via lactation

Table 89: Summary table of animal studies on adverse effects on lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Rat (SD) 12/sex/dose OECD TG 422 GLP Reliability 1 (according the to registration dossier)	**1-nitropropane** Purity: 99.69 % Inhalation (vapours) Doses: 0, 25, 50 and 100 ppm (corresp. to approx. 0, 0.092, 0.184 and 0.369 mg/L) Duration of exposure: 14d of premating period, during mating for both sexes and until gestation day 19 for females	**Maternal effects** Mortality: / Clinical signs: no effects observed BW: a trend to decrease was noted in males and was sign. lower at the highest at D7 of the premating period **Pups** Pup BW: sign. higher at 100 ppm in both sexes at lactation day 1 and 4 (within HCD range)	Anonymous 37, 2003

No human data or other relevant studies available.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

In a combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous 37, 2003), groups of 12 male and 12 female SD rats were exposed by inhalation (vapours) to 1-nitropropane (purity: 99.69 %) at a concentration of 0, 25, 50 or 100 ppm (approximatively equivalent to 0, 0.092, 0.184 and 0.369 mg/L, respectively). Females were exposed 14 d prior mating, during mating (2 w) and until the gestation day 19, whereas males were exposed 14 d prior mating and during mating (for a minimum exposure of 28 d). Each female was placed with one male from the same dose level.

The survival index was unaffected (see Table 90). At the highest dose, a significant higher pup body weight was noted in both sexes at D1 and D4 (see Table 91).

Table 90: Live births and survival index

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	-	-	-	-	

Table 91: Mean pups body weight (in g)

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

As the dams were exposed until gestational day 19 and sacrified on PND 5 and only early postnatal growth and survival rates data are available, relevance of this study to assess adverse effects on or via lactation is limited.

No EOGRTS, nor two-generation reproductive toxicity study nor combined repeated dose toxicity study with reproductive/developmental toxicity screening study was available for nitromethane and nitroethane.

10.10.9 Comparison with the CLP criteria

In the combined repeated dose toxicity with reproductive/developmental screening toxicity study (Anonymous 37, 2003), performed with 1-nitropropane, foetus were observed until the lactation day 4. The survival index was unaffected and the pups body weight increased at the highest (within in the HCD).

There is not enough data to conclude on effect on lactation as the dams were only exposed until GD19 and the pups observed until PND4.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Based on the available information, a classification as **Repr. 1B, H360Df (May damage fertility or the unborn child)** is warranted.

10.11 Specific target organ toxicity-single exposure

Hazard class not evaluated in this CLH dossier.

10.12 Specific target organ toxicity-repeated exposure

Table 92: Summary table of animal studies on STOT RE

Method, guideline, species, strain, sex, nb/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
1-NITROPROPANE			

Short-term repeated dose toxicity study Rat (SD) 5/sex/dose Japanese guideline GLP Reliability 1 (according to the registration dossier)	**1-nitropropane** Purity: > 98.5 % Oral (gavage) Doses: 0, 10, 30 and 100 mg/kg bw/d + 2 additionnal group 0 and 100 mg/kg bw/d (recovery group) Duration of exposure: 28 d Recovery period: 14 d	<u>100 mg/kg bw/d</u> *Males* 1 male killed in extremis at D27 (necropsy: dark kidneys, thickening of the forestomach and sloughing of the glandular gastric epith.) Decreased body weight compared to controls (-10 %) Increased salivation Increased brain weight (absolute and relative) *Females* Increased salivation Lower Hb, Ht values and erythrocyte count, higher clotting time Higher brain weight (absolute and relative) Increased kidney weight (absolute and relative) <u>30 mg/kg bw/d</u> *Males* No treatment-related effect in males *Females* Higher brain weight <u>10 mg/kg bw/d</u> No treatment-related effect in males and females **NOAEL: 30 mg/kg bw/d** **LOAEL: 100 mg/kg bw/d**	Anonymous 38, 1996

Range-finding study of the 28-day repeated dose toxicity study Rat (SD) 3/sex/dose	**1-nitropropane** Oral (gavage) Doses: 0, 10, 50, 150 and 250 mg/kg bw/d Duration of exposure: up to 14 d	250 mg/kg bw/d Mortality: all animals killed in extremis (maximum on D9) Clinical signs: ataxia, body tremors, pallor of extremities, loss of righting reflex, lethargy, decreased respiratory rate, ptosis, dehydratation, emaciation Gross pathology findings: pale kidneys, pale liver, pale adrenals, epithelial sloughting of the non-glandular stomach 150 mg/kg bw/d Mortality: one male killed in extremis on D7 Clinical signs: ataxia, body tremors, pallor of extremities, loss of righting reflex Gross pathology findings: pale kidneys, epithelial sloughting of the non-glandular stomach 50 mg/kg bw/d & 10 mg/kg bw/d No treatment-related effect **NOAEL: 50 mg/kg bw/d** **LOAEL: 150 mg/kg bw/d**	Anonymous 38, 1996

Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Rat (SD) 12/sex/dose OECD TG 422 GLP Reliability 1 (according to the registration dossier) *For males, +- 28 d exposure: Guidance value range for warranting classification as cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d* *cat. 1: C ≤0.6 mg/L/6 h/d* *For females: +- 45 d exposure, Guidance value range for warranting classification as cat. 2: : 0.4 < C ≤ 2 mg/L/6 h/d* *cat. 1: C ≤ 0.4 mg/L/6 h/d*	**1-nitropropane** Purity: 99.69 % Inhalation (vapours) Doses: 0, 25, 50 and 100 ppm (corresp. approx. to 0, 0.092, 0.184 and 0.369 mg/L) Duration of exposure: 6 h/d, 14 d of premating period, during mating for both sexes and until gestation day 19 for females 6 h/d, 7 d/w	Mortality: / Clinical signs: no effects observed <u>At 100 ppm (0.369 mg/L):</u> BW: tendency to ↓ in males (stat. sign. at day 7 of the premating period) Organ weight: in males: ↓ FBW and ↑ relative brain weight and relative testes weights Histopathology: multifocal degeneration of the olf. epith. (in 7 females); associated inflammation in 2 females <u>At 50 ppm (0.184 mg/L):</u> Histopathology: in females nasal tissue: inflammation and degeneration of the olf. epith. in 2 animals <u>At 25 ppm (0.092 mg/L):</u> No treatment-related effects **NOAEC: 25 ppm (0.184 mg/L)** **LOAEC: 50 ppm (0.369 mg/L)**	Anonymous 37, 2003
NITROMETHANE			

16-day repeated dose toxicity study Rat (F344) 5/sex/dose Non-GLP No guideline 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)	**Nitromethane** Purity: > 98 % Inhalation (vapours) Doses: 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.). Duration: 16 days, 6 h/d for 5 d/w	1500 ppm (3.750 mg/L) Sign. decreased BWG in males compared to controls *Nervous system*: Sciatic nerve degeneration in 5/5 males and 5/5 females *Respiratory tract*: Degeneration of the olf. epith. in 5/5 males and 5/5 females 750 ppm (1.880 mg/L) *Nervous system*: Sciatic nerve degeneration in 5/5 males and 5/5 females *Respiratory tract*: Degeneration of the olf. epith. in 5/5 males and 5/5 females 375 ppm (0.938 mg/L) *Nervous system*: Sciatic nerve degeneration in 5/5 males and 4/5 females *Respiratory tract*: Degeneration of the olf. epith. in 5/5 males and 5/5 females 188 ppm (0.47 mg/L) and lower No treatment-related effect in males and females **LOAEC: 375 ppm**	NTP, 1997

16-day repeated dose toxicity study Mouse (B6C3F1) 10/sex/dose Non-GLP No guideline 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)	**Nitromethane** Purity: > 98 % Inhalation (vapours) Doses: 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.). Duration: 16 days, 6 h/d for 5 d/w	1500 ppm (3.750 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; Increased absolute and relative liver weight in males and females 750 ppm (1.880 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; Increased absolute and relative liver weight in males and females 375 ppm (0.938 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; Increased absolute and relative liver weight in females. Increased relative liver weight in males. 188 ppm (0.47 mg/L) Increased absolute and relative liver weight in females 94 ppm (0.235 mg/L) Increased absolute and relative liver weight in females **LOAEC: 375 ppm**	NTP, 1997
13-week repeated dose inhalation toxicity study Rat (Fischer 344) 10/sex/dose Similar to OECD TG 413 GLP-compliance not specified Reliability 1 (according to the registration dossier)	**Nitromethane** Purity: > 98 % Inhalation (vapours) Doses: 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.). Duration: 13 weeks, 6 h/d for 5	1500 ppm (3.750 mg/L) Decreased FBW (-12 %) and BWG (-19 %) in males compared to controls *Nervous system*: Hindlimbs paralysis in 10/10 males and 10/10 females from day 21; Decreased hindlimb (males and females) and forelimb grip strength (only males); Sciatic nerve and spinal cord degeneration in 10/10 males and 10/10 females Startle response amplitude decreased in males and females	NTP, 1997

	d/w	*Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females + hyaline droplets in 8/10 males and 10/10 females Bone marrow hyperplasia in 10/10 males and 10/10 females Goblet cells hyperplasia in 10/10 males and 10/10 females Sign. decrease in T3, thyroxine and free thyroxine in both sexes at day 23 Sign. increase in erythrocytes and MetHb levels at week 13 Sign. decrease in the weight of left cauda, epididymis and testis <u>750 ppm (1.880 mg/L)</u> *Nervous system*: Sciatic nerve and spinal cord degeneration in 10/10 males and 10/10 females Startle response amplitude decreased in males and females *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; hyaline droplets in 4/10 females Bone marrow hyperplasia in 9/10 males and 7/10 females Sign. increase in erythrocytes and MetHb levels at week 13 <u>375 ppm (0.938 mg/L)</u> *Nervous system*: Sciatic nerve (5/10 males and 8/10 females) and spinal cord (9/10 males) degeneration Startle response amplitude decreased in males *Respiratory tract*: Degeneration of the olf. epith. in 9/10 males and 10/10 females Bone marrow hyperplasia in 6/10 females Sign. increase in erythrocytes and MetHb levels at week 13 <u>188 ppm (0.47 mg/L) and lower</u> Sign. increase in erythrocytes and MetHb levels at week 13 **LOAEC (systemic, male/female): 188 ppm (0.470 mg/L)** based on disturbance of hematological parameters **NOAEC (systemic, male/female): 94 ppm (0.235 mg/L)** **LOAEC (local, male/female): 375 ppm (0.938 mg/L)** for the upper respiratory tract **NOAEC (local, male/female): 188 ppm (0.470 mg/L)**	110

13-week repeated dose inhalation toxicity study Mouse (B6C3F1) 10/sex/dose Similar to OECD TG 413 GLP-compliance not specified Reliability 1 (according to the registration dossier)	**Nitromethane** Purity: > 98 % Inhalation (vapours) Doses: 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.). Duration: 13 weeks, 6 h/d for 5 d/w	1500 ppm (3.750 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; hyaline droplets in 10/10 males and 10/10 females *Spleen*: extramedullary hematopoiesis in 10/10 males and 9/10 females Increased absolute and relative kidney weight in females. Increased absolute and relative liver weight in males Sign. decrease in sperm motility (82.41 % v.s. 93.50 in controls) 750 ppm (1.880 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; hyaline droplets in 10/10 males and 10/10 females Increased absolute kidney weight in males and females. Increased absolute and relative liver weight in males Sign. decrease in sperm motility (86.47 % v.s. 93.50 in controls) 375 ppm (0.938 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; hyaline droplets in 10/10 males and 10/10 females Increased absolute kidney weight in males. Increased absolute and relative kidney weight in females. Increased relative liver weight in males Sign. decrease in sperm motility (85.09 % v.s. 93.50 in controls) 188 ppm (0.47 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 7/10 females; hyaline droplets in 1/10 males and 9/10 females Increased absolute kidney weight in males and females. 94 ppm (0.235 mg/L) No treatment-related effect in males and females	NTP, 1997

		LOAEC (systemic, male/female): 188 ppm (0.470 mg/L) based on modification of some organ weights **NOAEC (systemic, male/female): 94 ppm (0.235 mg/L)** **LOAEC (local, male/female): 375 ppm (0.938 mg/L)** for the upper respiratory tract **NOAEC (local, male/female): 188 ppm (0.470 mg/L)**	
Sub-chronic inhalation toxicity study Rat (SD) 50 males/dose Non-guideline Non-GLP Reliability 2 (according to the registration dossier)	**Nitromethane** Purity: 96.5 % Inhalation (vapours) Doses: 100 and 750 ppm (equivalent to 0.25 and 1.875 mg/L, respectively) Duration: 13 weeks and up to 24 weeks, 7 h/day for 5 d/week	750 ppm (1.875 mg/L) Decreased BWG compared to control from week 8. Decreased Ht, Hb and RBC from day 10 100 ppm (0.25 mg/L) No treatment-related effect **LOAEC (male): 745 ppm (1.875 mg/L)** based on decreased body weight gain after 2 months of exposure **NOEC (male): 98 ppm (0.25 mg/L)**	Lewis *et al.,* 1977

Sub-chronic inhalation toxicity study Rabbit (NZW) 15 males/dose Non-guideline Non-GLP Reliability 2 (according to the registration dossier, however doses at which effects were seen were not always clear)	**Nitromethane** Purity: 96.5 % Inhalation (vapours) Doses: 100 and 750 ppm (equivalent to 0.25 and 1.875 mg/L, resp.) Duration: 13 weeks and up to 24 weeks, 7 h/d for 5 d/w	<u>750 ppm (1.875 mg/L)</u> Reduced T4 levels at all time points Reduced Hb levels at 1-month Increased OCT levels at 1 and 3-month <u>100 ppm (0.25 mg/L)</u> Reduced T4 levels at all time points Reduced Hb levels at 1-month Increased OCT levels at 1 and 3-month Increased thyroid gland weights after 6-months of exposure, dose not specified. *Lung* : at 1-month, interstitial edema, moderate to moderately severe focal hemorrhage and sometimes necrosis in the area of hemorrhage. Frank edema in some animals. Dose not specified. **LOAEC (male): 100 ppm (0.25 mg/L)** based on reduced T4 levels throughout the study **No NOEC**	Lewis *et al.,* 1977

Sub-chronic oral repeated dose toxicity study Rat (albino) 10 males/dose Non-guideline Non-GLP Reliability 4 (according to the registration dossier)	**Nitromethane** Purity: unknown Oral (drinking water) Doses: 0, 0.1, 0.25 % (0.5, 1 and 2 %) Duration: 15 weeks	Doses starting from 0.5 % were not supported by the animals and therefore were abandoned after a week. <u>0.25 % (285 mg/kg bw/d)</u> 3/10 animals died Decreased body weight in surviving animals *Live*r: less stained and more granular liver cell cytoplasms, more lymphocytes in the periportal zone in 6/7 surviving animals *Spleen*: prominent Malpighian corpuscules in 2/7 surviving animals <u>0.1 % (150 mg/kg bw/d)</u> 4/10 animals died Decreased body weight in surviving animals *Liver*: enlarged hepatic cells in 2/6 surviving animals **LOAEL: 0.1 % (150 mg/kg bw/d)** **No NOAEL**	Weatherby *et al.,* 1955

2-year repeated dose inhalation toxicity study Equivalent or similar to OECD TG 451 GLP-compliant 2 years Mice (B6C3F1) 50/sex/group Reliability 1 (according to the registration dossier)	**Nitromethane** Purity > 99 % Impurities: 0.25 % nitroethane, 0.03 % 2-nitropropane Inhalation 6 h/d, 5 d/week Doses: 0, 188, 375 and 750 ppm (approx. equivalent to 0, 0.47, 0.94 and 1.87 mg/L, resp.)	*Mortality:* 38, 28, 40 and 42 % of M and 50, 44, 48 and 28 % of F exposed to 0, 188, 375 and 750 ppm, resp. *Clinical sign:* in the eyes, swelling and exophthalmos coincident with harderian gland tumours, in both sexes *BWG:* no effects in males, slightly increased BW in females during the study but similar to controls at study termination *Organ weights:* no data *Histopathology:* - sign. increased inc. olf. epith. degeneration in both sexes, in all treated groups - sign. increase inc. olf. epith. metaplasia in both sexes at 375 and 750 ppm - sign. increase inc. respiratory epith. hyaline degeneration in all treated groups in females and at the mid and high doses in males. *Neoplastic effects* **Harderian gland**: Male and female: Adenoma (%): M: 9/49 (18), 10/50 (20), 19/50 (38), 32/49 (65) F: 5/49 (10), 7/49 (14), 16/50 (32), 19/50 (38) Carcinoma (%): M: 1/49 (2), 1/50 (2), 6/50 (12), 5/49 (10) F: 1/49 (2), 2/49 (4), 4/50 (8), 3/50 (6) Adenoma or carcinoma (%): M: 10/49 (20), 11/50 (22), 25/50 (50), 37/50 (74) F: 6/49 (12), 9/49 (18), 20/50 (40), 21/50 (42)	NTP, 1997

		Liver: Female (%): Hepatocellular adenoma: F: 14/50 (28), 25/49 (51), 17/49 (35), 35/50 (70) Hepatocellular carcinoma: F: 10/50 (20), 14/49 (29), 8/49 (16), 12/50 (24) Hepatocellular adenoma or carcinoma: F: 19/50 (48), 34/49 (69), 22/49 (45), 40/50 (80) No increase in liver tumours was observed in Males. **Lung:** Male and female (%): Alveolar / bronchiolar 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) Alveolar / bronchiolar carcinoma M: 2/50 (4), 3/50 (6), 3/50 (6), 11/50 (22) F: 0/50 (0), 3/50 (6), 5/49 (10), 3/50 (6) Alveolar / bronchiolar adenoma or carcinoma M: 13/50 (26), 13/50 (26), 12/50 (24), 20/50 (40) F: 3/50 (6), 6/50 (12), 6/49 (12), 12/50 (24)	
NITROETHANE			

13-week repeated dose inhalation toxicity study Rat (Fischer 344) 15/sex/dose OECD TG 413 GLP: Study was initiated prior to GLP Reliability 2 (according to the registration dossier) Deviation: food consumption not assessed	**Nitroethane** Purity: > 97 % Impurities: Nitromethane < 1 %; 2-Nitropropane < 1.5 % Inhalation: vapours Doses: 0, 100, 350 and 1000 ppm (equivalent to 0, 0.3, 1.0 and 3.0 mg/L, resp.) Duration of exposure: 5/sex/dose for 30 d; 10/sex/dose for 92 d No recovery period, necropsy at the end of exposure period	<u>At 1000 ppm (3 mg/L)</u>: Decreased body weight gain Increased MetHb levels with cyanosis, Increased reticulocytes and Heinz bodies in peripheral blood Associated splenic congestion and extramedullary hematopoiesis Degenerative and inflammatory changes in the olfactory nasal epithelium, hepatocellular vacuolization, decreased cytoplasmic granularity of renal cortical tubular epithelium and ductal epithelial cells of the salivary glands <u>At 350 ppm (1 mg/L)</u>: Less severe changes in MetHb, spleen, nasal turbinates and salivary glands. <u>At 100 ppm (0.3 mg/L)</u>: Minimal changes in MetHb, spleen and salivary glands **LOAEC: 100 ppm**	Anonymous 26, 1982

13-week repeated dose inhalation toxicity study Mice (B6C3F1) 5/sex/dose OECD TG 413 Deviations: yes GLP: Study was initiated prior to GLP Reliability 1 (according to the registration dossier)	**Nitroethane** Purity: > 97 % Impurities: Nitromethane < 1 %; 2-Nitropropane < 1.5 % Inhalation: vapours Doses: 0, 100, 350 and 1000 ppm (equivalent to 0, 0.3, 1.0 and 3.0 mg/L, resp.) Duration of exposure: 93 d No recovery period, necropsy at the end of exposure period	At 1000 ppm (3 mg/L): Increased MetHb concentration including the increased presence of reticulocytes and Heinz bodies Moderate degeneration of the olfactory mucosa ± inflammation including moderate glandular hyperplasia Slight increase in cytoplasmic homogeneity of the liver Transient salivary gland alterations of decreased cytoplasmic granularity and decreased eosinophilic staining Presence of multinucleated spermatids in testes At 350 ppm (1 mg/L): Less extensive toxicity, only MetHb, nasal turbinates and liver affected At 100 ppm (0.3 mg/L): Minimal changes in nasal turbinates (females only) and transient effects (at 29 days not 13 weeks) on salivary glands **LOAEC: 100 ppm**	Anonymous 26, 1982
Range-finding study for 13-weeks repeated dose inhalation toxicity study Rat (Fischer 344) 5/sex/dose GLP: Study was initiated prior to GLP and completed with GLP	**Nitroethane** Purity: unknown Inhalation: vapours Doses: 0, 350, 1000, 2000 or 4000 ppm (equivalent to 0, 1.0, 3.0, 6.0 or 12 mg/L, resp.) Exposure period: 4 d	Please refer to chapter 10.3 (Inhalation acute toxicity study, 4-day study in rats) All animals died at the highest dose: probable cause: hypoxia secondary to methemoglobinemia Specific toxicity from 350 ppm: - cyanosis, a manifestation of the MetHb effect determined in the 13-week study - hyperemia of the nasal turbinates **LOAEC: 350 ppm**	Anonymous 26, 1982

Chronic inhalation toxicity study 2 years Similar to OECD TG 453 GLP compliant: not specified Rat (Long-Evans) 40/sex/group (control & 100 ppm) 41 males & 39 females (200 ppm) Reliability 2 (according to the registration dossier) Major deviations: - only 2 doses tested - 40 animals / group - some tissues were not examined microscopically (parathyroid, caecum, rectum, bone marrow,…)	**Nitroethane** Purity: 97.92 % Impurities: nitromethane 0.01 % and 2-nitropropane 2.07 % Inhalation 7 h/d, 5 d/w Conc.: 0, 100, 200 ppm (corresp. approx. to 0, 0.31 and 0.61 mg/L, resp.)	*Mortality:* no treatment-related effect *BW*: sign. ↓ at 100 ppm in males and at 200 ppm in females *Clinical chemistry:* slight but sign. ↑ of total protein and BUN in females exposed to 200 ppm *Hematology*: No effects observed. MetHb level not reported. *Organ weights (brain, liver, kidneys, lungs, heart):* no treatment-related effect *Histopathology*: no effect *Neoplastic effects:* No treatment-related increase of tumours In all animals (controls and treated groups), high incidence of benign tumours (adenoma of the pituitary gland) Very rare malign tumours, not treatment-related No HCD available	Anonymous 35, 1986

Table 93: Summary table of human and other studies relevant for STOT RE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Case study report	**Nitroethane** Purity: 100 % Oral exposure Quantity < 1 ounce (less than 30 mL)	Human 1 boy 20-month old	Cyanosis Methemoglobinemia level: increased to 39 % Full recovery after intravenous methylene blue injection	Hornfeldt and Rabe, 1994
Case study report	**Nitroethane** Purity: 100 % Oral exposure Quantity: max. 90 mL	Human 1 girl 13-month old	Cyanosis, tachypnea, lethargy, emesis 7 h after ingestion. Methemoglobinemia up to 53 % 23 h after ingestion.	Osterhoudt *et al.*, 1995

Disregarded study **Neurotoxicity study** No guideline Reliability 4 (according to the registration dossier) GLP: not specified Rat SD Male/female 4-5 animals in each group	**Nitroethane** Purity: unknown 275 mg/kg Oral: gavage Two hours after a single acute oral dose of nitroethane, the profile of several neurochemicals in the brain was examined.	Disregarded study: origin of the effects are not described (direct/indirect effect due to hypoxia)	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	Kanada *et al*., 1994
Hepatotoxicity No guideline GLP: not specified Reliability 2 (according to the registration dossier) BALB/c mice Male/female: 19-25 g 3-5/sex/dose	**Nitroethane** Purity: unknown 4.5, 6.7 or 9.0 mmol/kg IP	Reporting deficiencies (doses not clearly stated for example)	No sign. increase in SDH, ALT or AST activity. No significant abnormalities in livers of mice exposed to 9 mmol/kg	Dayal R *et al.,* 1989

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Data on 1-Nitropropane

Oral

In a short term repeated dose toxicity study (Anonymous 38, 1996), groups of 5 male and 5 female SD rats were given daily by gavage 1-nitropropane (purity: > 98.5 %) at a concentration of either 0, 10, 30 or 100 mg/kg bw/d during 28 days. Additionally, 2 satellite groups received by gavage 1-nitropropane at a concentration of either 0 or 100 mg/kg bw/d during 28 days and were observed during 14 days (recovery period).

One male of the highest dose was killed in extremis at the day 27. The necropsy of this animal revealed dark kidneys, thickening of the forestomach and sloughing of the glandular gastric epithelium. The remaining animals (both sexes) of the high dose level showed an increased incidence of salivation. Moreover, a slight body weight decrease was noted in males at this dose level (see Table 94). This change was not observed in males of the recovery group or in females. Final body weight was 329, 333, 365 and 292 g for males and 231, 243, 235 and 227 g for females at 0, 10, 30 and 100 mg/kg bw/d, respectively for the main groups. For the satellite groups, final body weights were 391 and 385 for males and 259 and 250 g for females at 0 and 100 mg/kg bw/d, respectively.

Table 94: 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

Significantly lowered hemoglobin and hematocrit values, erythrocyte count and significantly lowered white blood cell count were observed in females of the highest dose. In males, the methemoglobin was significantly increased at the low group and only slightly increased at the highest dose. In females, a tendency to increase was observed in all tested groups (dose-dependent). Furthermore, higher clotting time was observed in females and lower platelet count was noted in males (see Table 95).

Table 95: 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
MetHb (%)	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

At necropsy, the final body weight did not exhibit significant treatment-related changes (329, 333, 365 and 292 g respectively at 0, 10, 30 and 100 mg/kg bw/d for main groups and 391 and 385 g respectively at 0 and 100 mg/kg bw/d for satellite groups in males and 231, 243, 235 and 227 g respectively at 0, 10, 30 and 100 mg/kg bw/d in main groups and 259 and 250 g respectively at 0 and 100 mg/kg bw/d in satellite groups in females).

Examination of organ weight revealed few changes. In males, animals exposed to 100 mg/kg bw/d (main group) exhibited a statistically significantly higher absolute brain weight (1.9961, 2.0477, 1.9955 and 2.0775* g at 0, 10, 30 and 100 mg/kg bw/d, respectively in main groups and 1.9952 and 2.0260 g at 0 and 100 mg/kg bw/d, respectively in satellite groups) and a statistically significantly lower absolute pituitary weight (0.0091, 0.0102, 0.0103 and 0.0072* g at 0, 10, 30 and 100 mg/kg bw/d, respectively in main groups and 0.0105 and 0.0096 g at 0 and 100 mg/kg bw/d, respectively in satellite groups). The relative brain weight was also statistically significantly higher (0.6076, 0.6189, 0.5515 and 0.7169** g at 0, 10, 30 and 100 mg/kg bw/d, respectively in main groups and 0.5126 and 0.5297g at 0 and 100 mg/kg bw/d, respectively in satellite groups). Whereas in females, a statistically significantly higher absolute brain weight was noted in animals of the mid and high dose levels (1.8593, 1.8909, 1.9453* and 2.0206*** g at 0, 10, 30 and 100 mg/kg bw/d, respectively in main groups and 1.9062 and 1.8947 g at 0 and 100 mg/kg bw/d, respectively in satellite groups) (relative weight inaffected). Moreover, animals exposed to the highest dose exhibited a statistically significantly higher kidneys weight (1.6071, 1.6922, 1.6761 and 1.7762* g at 0, 10, 30 and 100 mg/kg bw/d, respectively in main groups and 1.6930 and 1.7471 g at 0 and 100 mg/kg bw/d, respectively in satellite groups). The relative kidneys weight was also significantly higher in the main group, at the highest dose. A slight decrease in ovary weight was observed at the highest dose (0.1259, 0.1264, 0.1273 and 0.1073g at 0, 10, 30 and 100 mg/kg bw/d, respectively in main groups and 0.1359 and 0.1207g at 0 and 100 mg/kg bw/d, respectively in satellite groups). The relative ovary weight was also significantly lowered at the highest dose, in the main group. However, the microscopic examination did not reveal treatment-related effects. This study is taken into account for classification since the tested doses are in line with the guidance dose range relevant for classification. Effects seen on the hematological system are consistent with effects seen with nitromethane (e.g. reduced hemoglobin levels in the 13-week repeated dose inhalation toxicity study NTP, 1997) and potentially explain the pale foetuses reported in Anonymous 36 (2017).

The LOAEL was determined to be 100 mg/kg bw/d due to brain weight and blood effects; the NOAEL was therefore set at 30 mg/kg bw/d. The guidance value range for warranting classification as STOT RE cat. 2 is > 30 and ≤ 300 mg/kg bw/day. The DS notes that all doses are relevant for classification.

In the range-finding of the 28-day repeated dose toxicity study (Anonymous 38, 1996), groups of 3 male and female SD rats were exposed by gavage to 1-nitropropane at a concentration of 0, 10, 50, 150 and 250 mg/kg bw/d up to 14 days.

Mortality was noted at 150 and 250 mg/kg bw/d. At 150 mg/kg bw/d, one male was killed in extremis on D 7, while at 250 mg/kg bw/d, all animals were killed in extremis (2 females on D 4, 1 male on D 6 and the remaining on D 9). Severe clinical signs were noted at the 2 highest doses (pallor of the extremities, ataxia, body tremors, loss of righting reflex at 150 and 250 mg/kg bw/d and lethargy, decreased respiratory rate, emaciation, ptosis and dehydration at 250 mg/kg bw/d). Furthermore, lower body weight was observed at the highest dose at D 4 and D 8. Necropsy revealed findings at the 2 highest doses, such as pale kidneys, pale liver (only at 250 mg/kg bw/d), pale adrenals (only at 250 mg/kg bw/d) and epithelial sloughing of the non-glandular region of stomach. Histopathology was not performed.

The LOAEL was determined to be 150 mg/kg bw/d due to neurological effects; the NOAEL was therefore set at 50 mg/kg bw/d. The guidance value range for warranting classification as STOT RE cat. 2 is > 60 and ≤ 600 mg/kg bw/day.

Inhalation

In a combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous 37, 2003), groups of 12 male and 12 female SD rats were exposed by inhalation (vapours) to 1-nitropropane (purity: 99.69 %) at a concentration of either 0, 25, 50 or 100 ppm (corresponding to approx. 0, 0.092, 0.184 and 0.369 mg/L, respectively). Females were exposed 14 d prior mating, during mating (2 w) and until the gestation day 19, whereas males were exposed 14 d prior mating and during mating (for a minimum exposure of 28 d). Each female was placed with one male from the same dose level.

As mentioned in chapter 10.10.2, all animals survived during the exposure period and did not exhibit clinical signs. A trend to lower body weight value was observed in males exposed to the highest dose while body weight was not significanty affected in females (see Table 60 and Table 61). At necropsy, organ weights were examined and revealed few significant changes (see Table 62). Indeed, in males exposed to 100 ppm showed a statistically significantly reduced final body weight value (354.1, 358.8, 357.3 and 328.7* g at 0, 25, 50 and 100 ppm, respectively) as well as a statistically significantly higher relative brain weight (0.562, 0.567, 0.572 and 0.622* g/100 g at 0, 25, 50 and 100 ppm, respectively) and relative testes weight (0.867, 0.902, 0.846 and 0.965* g/100 g at 0, 25, 50 and 100 ppm, respectively). Organ weights in females were not significantly changed. Histopathological examination revealed effects in females nasal tissue (such as multifocal degeneration of the olfactory epithelium, sometimes with signs of inflammation) (see Table 63).

The LOAEC was determined to be 50 ppm due to effects seen in the nasal tissue, the NOAEC was therefore set at 25 ppm. Males and females were not exposed for the same amount of days. The guidance values range relevant for classification are therefore not identical. For males, exposed for approximatively 28 days, the guidance values range for warranting classification as cat. 2 is 0.6 < C ≤ 3 mg/L/6h/d and cat. 1 is C ≤0.6 mg/L/6h/d. For females exposed approximatively for 45 days, the guidance values range for warranting classification as cat. 2 is 0.4 < C ≤ 2 mg/L/6h/d and as cat. 1: C ≤ 0.4 mg/L/6h/d. The concentrations used here (0, 25, 50 or 100 ppm) are equivalent to 0, 0.092, 0.184 and 0.369 mg/L, respectively, for 1-nitropropane. In males and in females, the highest dose used is therefore relevant for classification cat. 1.

Case report

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Data on Nitromethane

Oral exposure

In a sub-chronic repeated dose toxicity study (Weatherby *et al.*, 1955), groups of 10 male and 10 female albino rats were orally exposed to nitromethane in drinking water for 15 weeks. Doses chosen were 0, 0.1, 0.25, 0.5, 1 and 2 % but doses starting from 0.5 % were not supported by the animals and therefore were abandoned after a week. Only the control and 0.1 and 0.25 % groups were kept, corresponding to an average

daily intake of 150 and 285 mg/kg bw/day nitromethane, respectively. Moreover, 4 and 3 animals out of 10 died in groups exposed to 0.1 and 0.25 %, respectively.
In surviving animals, necropsy was performed and tissues examined. A the end of exposure period, gross and microscopic changes were assessed in the heart, lungs, liver, spleen, kidney, testes, adrenal gland and small intestine.
Decreased body weight was noted in surviving animals at 0.1 and 0.25 % (no more information available). Histopathological findings indicated larger hepatic cells with a prominent nucleus in 2/6 surviving animals in the 0.1% group exposed to 0.1 % nitromethane. In the 0.25 % group, 2/7 surviving animals had more prominent Malpighian corpuscles compared to normal spleen. In 6/7 animals, the liver cells cytoplasms were less stained and more granular compared to control group, and more lymphocytes were noted in the periportal zone.
All animals in the control group survived, 1/10 rats had large hepatic cells with prominent nuclei.

This study is considered not relevant for classification because the tested doses are above the CLP guidance dose range relevant for STOT RE classification.

Inhalation

In a 16-day repeated dose toxicity study (NTP, 1997), groups of 5 male and 5 female rats were daily exposed to 0, 94, 188, 375, 750 or 1500 ppm (equivalent to 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L, respectively) nitromethane by inhalation for 6 h + 12 min during 16 days. All animals survived until the end of the study. The mean body weight gain of male rats in the 1500 ppm was slightly but statistically significantly less than that of controls whereas no difference was noted in the body weight and body weight changes in females. In the highest dose group, all male and female rats demonstrated hypoactivity and a loss of coordination in the hindlimbs near the end of the study. Other clinical signs in this group included preening, rapid breathing and hyperactivity early in the study. The relative liver weights of all exposed groups of male rats and the absolute and relative liver weights of females exposed to 375 ppm or greater were significantly superior than those of controls.

Sciatic nerve degeneration and minimal to mild degeneration of the olfactory epithelium was observed in the nose of males and females exposed to 375 ppm and above. Also rats exposed to 750 or 1500 ppm had reduced myelin around sciatic axons.

Table 96: histopathological data

Dose level (in ppm)	0	94	188	375	750	1500
Males						
Nb animals examined	5	5	5	5	5	5
Degeneration olf. epith.	0	0	0	5** (minimal)	5** (mild)	5** (mild)
Sciatic nerve degeneration	0	0	0	5** (minimal)	5** (mild)	5** (moderate)
Females						
Nb animals examined	5	5	5	5	5	5
Degeneration olf. epith.	0	0	0	4** (minimal)	5** (mild)	5** (mild)
Sciatic nerve degeneration	0	0	0	5** (minimal)	5** (mild)	5** (moderate)

For a 16-day study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: $\leq$ 1.2 mg/L/d for Cat. 1 and 1.2 $\leq$ C $\leq$ 6 mg/L/d for Cat. 2, respectively. The dossier submitter considers therefore this 16-day repeated dose toxicity study as relevant for STOT RE classification. Nonetheless, the DS questions the selection of doses in this study that might have been too low. Indeed, uncertainty remains about the severity of the effets at a higher dose. Calculated doses for a shorter study via the Haber's rule may lead to unclear relevance of the effects. However, the DS notes that the early onset of neurological and respiratory effects can be supportive of a classification for STOT RE (nervous system and respiratory tract).

In another 16-day repeated dose toxicity study (NTP, 1997), groups of 5 male and 5 female mice were daily exposed to 0, 94, 188, 375, 750 or 1500 ppm (equivalent to 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L/6 h/day, respectively) nitromethane by inhalation for 6 h plus 12 minutes during 16 days. All animals survived until the end of the study.The final mean body weights and mean body weight gains of exposed males and females were similar to those of controls. Clinical findings included hypoactivity and tachypnea in male and female mice in the high dose group near the end of the study.

The absolute and relative liver weights of male mice in the 750 and 1500 ppm groups and female mice in all exposed groups were significantly greater than those of the controls. The relative liver weight of males in the 375 ppm group was also significantly greater than that of the controls.

Degeneration of the olfactory epithelium of the nose was observed microscopically in all males and females exposed to 375 ppm or greater. This lesion was of minimal severity in males and minimal to mild severity in females.

For a 16-day study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 1.2 mg/L/d for Cat. 1 and 1.2 ≤ C ≤ 6 mg/L/d for Cat. 2, respectively. The dossier submitter considers therefore this 16-day repeated dose toxicity study as relevant for STOT RE classification.

In a 13-week repeated dose inhalation toxicity study (NTP, 1997), groups of 10 male and 10 female Fischer 344 rats were exposed to nitromethane during 6-h per day, for 5 d/week during 13 weeks. Doses chosen were 0, 94, 188, 375, 750 and 1500 ppm corresponding to 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L, respectively. 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 D3 and D23). At the termination of the study, all rats from the "core study" were also necropsied for clinical pathology evaluation.

Statistically significant decreases in final body weight (-12 %) and body weight gain (-19 %) were reported in males exposed to 1500 ppm, in comparison with controls.

Table 97: BW and BWG (in g)

Exposure level (ppm)		0	94	188	375	750	1500
♂	N	10	10	10	10	10	10
	BW at start	107 ± 3	105 ± 2	113 ± 2	109 ± 3	106 ± 2	109 ± 2
	FBW	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**
♀	N	10	10	10	10	10	10
	BW at start	95 ± 1	96 ± 2	97 ± 2	95 ± 2	96 ± 2	94 ± 2
	FBW	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

Neurobehavioral evaluation showed hindlimbs paralysis in all rats exposed to 1500 ppm, in both sexes, starting from day 21; as well as in 1 male and 4 females at 750 ppm, starting on D 63. Concerning grip strength, it was significantly decreased in males at 1500 ppm (both in hindlimbs and forelimbs) and at 750 and 1500 ppm in females (only hindlimbs). Startle response amplitude (in volt) tended to decrease in males starting from 375 ppm and above and in females beginning at 750 ppm and above.

Hematological results showed a dose-related significant increase in MetHb concentrations in both sexes and a significant decrease in Ht and Hb levels starting from 375 ppm in males and 188 ppm in females. As shown

in the same table, decrease in T3, thyroxine and free thyroxine in animals exposed to 1500 ppm, in both sexes, significant at day 23 and slightly decreased after 13 weeks of exposure.

Table 98: Hematological and biochemistry findings

	Dose level (ppm)	0	94	188	375	750	1500
In males							
D 3	N	10	10	10	10	10	10
D 23	N	6	8	9	10	10	10
Week 13	N	10	10	10	10	10	10
D 3	Htc (%)	36.7	36.3	35.2*	33.1**	31.7**	32.3**
D 23		40.7	43.2	40.4	37.6*	34.0**	30.3**
Week 13		46.3	46.6	46.1	44.6**	42.5**	39.2**
D 3	Hb (g/dL)	13.9	13.5	13.3*	12.6**	12.2**	12.4**
D 23		15.3	16.1	15.0	14.3*	13.2**	11.9**
Week 13		15.3	15.4	15.2	14.8**	14.3**	13.4**
D 3	Erythrocytes (10^6/µl)	7.75	7.58	7.38**	7.16**	6.97**	6.94**
D 23		8.74	9.37	9.00	9.36*	9.1	7.77
Week 13		9.12	9.43**	9.53**	9.72**	10.10**	9.41**
D 3	MetHb (g/dL)	0.16	0.14	0.19	0.34**	0.21*	0.22*
D 23		0.08	0.06	0.08	0.16	0.15*	0.28**
Week 13		0.15	0.17	0.17*	0.17*	0.21**	0.41**
D 23	T3 (ng/mL)	116	105	105	91**	95*	92*
Week 13		123	134	125	138	137	134
D 23	Thyroxine (µg/dL)	5.4	5.2	5.2	4.4*	5.0	4.4**
Week 13		4.9	5.2	5.1	5.3	5.2	5.9**
D 23	Free thyroxine (ng/dL)	1.3	1.2	1.2	0.9**	1.1*	1.0*
Week 13		1.4	1.4	1.2	1.2	1.3	1.5
In females							
3	N	10	10	10	10	10	10
23	N	10	10	10	10	10	8
Week 13	N	10	10	10	10	10	10
3	Htc (%)	38.9	38.7	38.1	36.7**	36.0**	36.6**
23		42.6	40.5**	41.1*	37.9**	35.3**	31.7**
Week 13		46.8	46.6	44.7**	44.4**	40.7**	37.8**
3	Hb (g/dL)	14.9	14.9	14.6	14.0**	13.7**	14.1**
23		16.2	15.4**	15.6*	14.5**	13.5**	12.5**
Week 13		16.0	15.8	15.3**	15.3**	14.1**	13.4**
3	Erythrocytes (10^6/µl)	8.39	8.42	8.34	8.10	7.87**	8.14*
23		9.03	8.86	9.35	9.32	9.14	8.16
Week 13		8.71	8.91	8.92	9.42**	9.24**	8.51
3	MetHb (g/dL)	0.20	0.27	0.17	0.10*	0.11	0.16
23		0.09	0.10	0.12*	0.12**	0.19**	0.35**
Week 13		0.20	0.20	0.20	0.21	0.25**	0.40**
23	T3 (ng/mL)	110	107	109	96	92*	85**
Week 13		150	148	163	152	148	136
23	Thyroxine (µg/dL)	4.8	4.6	4.1*	3.6**	3.3**	3.2**
Week 13		4.6	4.1	4.3	4.0	3.7	4.0
23	Free thyroxine (ng/dL)	0.9	1.1	0.9	0.7	0.5**	0.5**
Week 13		0.9	0.7	0.7	0.7	0.6	0.7

Histopathological findings included bone marrow hyperplasia from 375 ppm in females and from 750 ppm in males increasing in a dose-dependant way. Sciatic nerve and spinal cord degeneration were also reported 375

ppm in males and females showing a dose-dependancy trend as well. Local effects included degeneration of the olfactive epithelium and hyaline droplets in males and females from 375 ppm.

Table 99: Histopathological findings

Exposure level (ppm)		0	94	188	375	750	1500
♂	**N**	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**
♀	**N**	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**

The LOAEC (systemic, male/female) was determined as 188 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.

For a 90-d study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 0.2 mg/L/d for Cat. 1 and 0.2 ≤ C ≤ 1 mg/L/d for Cat. 2, respectively. The dossier submitter considers therefore this repeated dose toxicity study as relevant for STOT RE classification until the dose of 375 ppm. Hematological findings were reported at all time points (day 3, day 23 and week 13) starting at doses from 375 ppm. Their early onset increases the confidence in the severity of these hematological effects. Furthermore, significant increased incidence of degeneration of the olf. Epith was also observed at doses ≥375 ppm.

In a 13-week repeated dose toxicity study (NTP, 1997), groups of 10 male and 10 female B6C3F1 mice were exposed by inhalation to 0, 94, 188, 375, 750 and 1500 ppm (equivalent to 0, 0.235, 0.470, 0.938, 1.880 and 3.750 mg/L, respectively) nitromethane during 6-h per day, for 5 d/week during 13 weeks. Clinical signs and body weight were observed weekly. Additional groups of 5 mice per sex were included before the starting of the study for parasite and clinical pathology assessment 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 reported on body weight and body weight changes at any dose. In males, a significant increase of the relative liver weight starting at 375 ppm and of absolute right kidney weights (except at 1500 ppm), in comparison with the controls was observed. In females, a significant increase of the relative and absolute weights of kidneys at 750 and 1500 ppm, in comparison with the controls, was reported.

Olfactory epithelial degeneration and respiratory epithelial hyaline droplets were observed microscopically in all male and female mice exposed to 375 ppm or greater. Moreover, 7 females in the 188 ppm also had epithelial degeneration. Finally, 1 male and 9 females in the 188 ppm groups and 2 females in the 94 ppm group had hyaline droplets.

At 1500 ppm, all males and 9 females had extramedullary hematopoiesis of the spleen. Although this lesion was also observed in a few males and females exposed to 375 ppm or 750 ppm, the incidences were very low (0, 1, 0, 1, 2 and 10 ** out of 10 males and 0, 0, 0, 2, 3 and 9 out of 10 females exposed to 0, 94, 188, 375, 750 and 1500 ppm, respectively). No kidney, liver or lung lesions were observed in exposed mice.

Table 100: Histopathological findings

Exposure level (ppm)		0	94	188	375	750	1500
♂	**N**	10	10	10	10	10	10
	Degeneration olf. epith.	0	0	0	10**	10**	10**
	Hyaline droplets, olf. epith.	0		1	10**	10**	10**
	Extramedullary Hematopoiesis, spleen	0	1	0	1	2	10**
♀	Degeneration olf. epith.	0	0	7**	10**	10**	10**
	Hyaline droplets, olf. Epith.	0	2	9**	10**	10**	10**
	Extramedullary Hematopoiesis, spleen	0	0	0	2	3	9**

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 ppm on organ weights, the LOAEC (local, male/female) was 375 ppm for the upper respiratory tract, and the NOAEC (local, male/female) was 188 ppm.

For a 90-d study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 0.2 mg/L/d for Cat. 1 and 0.2 ≤ C ≤ 1 mg/L/d for Cat. 2, respectively. The dossier submitter considers therefore this repeated dose toxicity study as relevant for STOT RE classification until the dose of 375 ppm. Doses of 750 and 1500 ppm are outside the CLP guidance range for STOT RE classification.

In another sub-chronic inhalation repeated dose toxicity study (Lewis *et al.*, 1977), male rats were exposed by inhalation to 100 and 750 ppm nitromethane (equivalent to 0.25 and 1.875 mg/L, respectively) 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).

Starting from the 8th week, a decrease in BWG was observed in rats exposed to 750 ppm, in comparison with the control group. The decrease was significant except during week 13. No effect on body weight was noted in rats exposed to 100 ppm, compared to controls (no raw data available).

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 day10, 1-month and 3-month time points. The difference with the control group was not significant only at the day10 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. There were no treatment-related effects in methemoglobin and prothrombin concentrations.

Table 101: Hematological parameters

Parameters	Dose level (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 * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; results at 100 ppm are not available

Ornithine carbamyl transferase (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.

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.

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.

The **LOAEC (male) was 745 ppm** based on a decrease in body weight gain after 2 months of exposure and the **NOEC was 98 ppm**.

For a 90-d study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 0.2 mg/L/d for Cat. 1 and 0.2 ≤ C ≤ 1 mg/L/d for Cat. 2, respectively. The dossier submitter considers therefore this repeated dose toxicity study as relevant for STOT RE classification with the dose of 100 ppm. Dose of 750 ppm is outside the CLP guidance range for STOT RE classification and the selection of doses may have been inappropriate.

In a rabbit sub-chronic inhalation repeated dose toxicity study (Lewis *et al.*, 1977), males were exposed to 100 and 750 ppm nitromethane (equivalent to 0.25 and 1.875 mg/L, respectively) for 13 weeks, and up to 24

weeks. A clinical examination as well as blood testing and histopathological assessment were performed at various time points (1, 3 and 6 months).

No mortality occurred and no effects on body weight or body weight changes were noted during the study. Hemoglobin levels were reduced at 1 month. No effects were seen on the erythrocytes count, hematocrit, methemoglobin and prothrombin levels. 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.

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. 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.

The LOAEC (male) was 98 ppm based on reduced T4 levels throughout the study.

For a 90-d study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 0.2 mg/L/d for Cat. 1 and 0.2 ≤ C ≤ 1 mg/L/d for Cat. 2, respectively. The dossier submitter considers therefore this repeated dose toxicity study as relevant for STOT RE classification with the dose of 100 ppm. Dose of 750 ppm is outside the CLP guidance range for STOT RE classification and doses selection might have been inappropriate.

In a 2-year study in rats (NTP, 1997), Fisher F344/N male and female rats were exposed during 2 years to vapours of nitromethane at doses of either 0, 94, 188 or 375 ppm (6 hours/day, 5 days/week). The doses of 0, 94, 188 and 375 ppm were approximatively equivalent to 0, 0.235, 0.47 and 0.94 mg/L, respectively.

Mortality was relatively high in all dose groups, in both sexes, but was not dose-related (see Table 41). Body weights were not affected in males but they were slightly higher than in controls in females exposed to 375 ppm (see Table 42). Masses on shoulders 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.

At necropsy, in females, the incidences of fibroadenoma, fibroadenoma or adenoma (combined) and of fibroadenoma, adenoma or carcinoma (combined) of the mammary gland increased in a dose-dependent manner, confirming clinical observations (see Table 43).

For a 2-y study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 0.025 mg/L/d for Cat. 1 and 0.025 ≤ C ≤ 0.125 mg/L/d for Cat. 2, respectively. Therefore the data presented here are supportive information.

In a 2-year study in mice (NTP, 1997), B6C3F1 male and female mice were exposed during 2 years to vapours of nitromethane at doses of either 0, 188, 375 or 750 ppm (6 hours/day, 5 days/week). The doses of 0, 188, 375 and 750 ppm were approximatively equivaent to 0, 0.47, 0.94 and 1.87 mg/L, respectively.

Mortality tended to be high in all dose groups, in both sexes, but the survival rate of females exposed to the highest dose was marginally greater than in other groups (see Table 46). Body weight gains were not affected by the treatment in males. In females, mean BW were similar in all dose groups at study termination (see Table 47). Coincidently with a swelling around the eyes and exophthalmos in exposed animals of both sexes, neoplasms of the Harderian gland were observed (see Table 49).

Histopathological findings show that nasal lesions were increased in exposed animals of both sexes (Table 48). Indeed, a significant dose-dependent increase in olfactory epithelium degeneration was observed at 188,

375 and 750 ppm, in both sexes. Tumours incidence in the Harderian gland, the liver and the lung are presented in Table 49. Liver tumours were seen only in females: 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 well.

For a 2-y study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 0.025 mg/L/d for Cat. 1 and 0.025 ≤ C ≤ 0.125 mg/L/d for Cat. 2, respectively. Therefore the data presented here are supportive information.

Case reports

/

Data on Nitroethane

Oral

/

Inhalation

In a sub-chronic repeated dose toxicity study (Anonymous 26, 1982), 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/wk for a total of 64-65 exposures (over a 92-d period) with an interim sacrifice of rats after 20-21 exposures (over a 30-d period).

Parameters monitored were clinical observations, body weights, organ weigths, hematological characteristics including methemoglobin (MetHb) determination, clinical chemistries, urinalysis, gross pathology and histopathology.

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 methemoglobin levels, spleen, nasal epithelium and salivary glands. The changes were minimal at 100 ppm in the methemoglobin level, spleen and salivary glands.

No death occurred during the experiment.

Growth retardation was reported in the 1000 and 350 ppm in female and male rats. 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 102: Rat Body weights in a 13 weeks inhalation toxicity study (in g)

0	100	350	1000	Exposure level (ppm)		0	100	350	1000
				Exposure	Experiment				

Males				day	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*

Two clinical findings, cyanosis and red eyes, were consistent with the grossly observable treatment-induced methemaglobinemia.

- Dull, dark red eyes were very pronounced in the 1000 ppm group (appeared after the first exposure and thereafter), while ot was not very distinctive in the 350 ppm group (appeared after 4 weeks of exposure).

- Grayish or bluish colored skin of the extremities (cyanosis) was reported in the 350 ppm group after 9 weeks of exposure and in the 1000 ppm group after 4 exposure and thereafter. Effects disappeared within 19 hours after exposure, in both treatment groups.

- 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 occured in this laboratory and were not judged to be treatment-related.

Prior to interim kill (20th exposure day, D29 of the experiment), methemoglobin was dosed in blood, 15 hours after the last exposure (Part A of Table 103). 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 methemoglobinemia 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 Table 103).

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 Table 103).

At terminal kill, a time-sequenced analyse (Part D of Table 103) was performed less than 30 min after exposure, 4 and 19h 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 103: Methemoglobinemia

Males					**Females**			
0	100	350	1000	Dose levels	0	10	350	1000
A: 15 hours after the 20th exposure								
5	5	5	5	N	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								
-	-	-	-	N	5	-	-	5
-	-	-	-	MetHb	0.6±0.5	-	-	57.4*±5.2
C: immediately after the 30th exposure								
5	5	5	5	N	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	N	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

Prior to the interim kill (30 days), statistically significant lowered hemoglobin values in male rats and statistically significant increases of the WBC counts were seen in the 1000 ppm group. At 350 and 1000 ppm, increased levels of reticulocytes and Heinz bodies were noted.

Prior to the terminal kill (92 days), a statistically significant increased PCV and a decreased RBC count was noted in females as well as statistically significant lowered hemoglobin values in male rats, at 1000 ppm. At 350 and 1000 ppm, increased levels of reticulocytes and Heinz bodies were noted.

Table 104: Haematological 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	Reticulocytes	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	Reticulocytes	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 (%); *p<0.05

Histological assessment is described in Table 105 below. Degeneration and inflammation of the olfactory epithelium was reported in males and females exposed to 350 and 1000 ppm, at interim and terminal sacrifice.

Table 105: Histopathological assessment

	Males				**Females**			
Dose levels (ppm)	0	100	350	1000	0	100	350	1000
At interim sacrifice (D30)								
N	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 inflam. myocardium	0	3	0	0	0	0	0	0
Slight multifocal inflam. myocardium	0	0	0	0	0	1	0	0
Slight Focal subacute inflam.	1	0	0	0	0	0	0	0
Slight Focal subacute myocardial inflam.	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								
N animals	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. Subpleural area	1	0	0	1	1	0	0	0
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 infla. 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 infla. Olf. Epith	0	0	0	4	0	0	2	5
Moderate diffuse chronic active infla. 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 resp. epith.	1	0	0	0	0	0	0	0

CJ= corticomedullary junction

The LOAEC was set at 100 ppm for males and females based on histopathologic changes in the salivary gland after 13 weeks exposure and extramedullary hematopoiesis starting from interim kill in males and observed in all males, at all doses at terminal kill.

This study is considered relevant for classification because the tested doses are in line with the guidance dose range relevant for classification (up to 350 ppm = 1 mg/L).

In a sub-chronic repeated dose toxicity study (Anonymous 26, 1982), groups of B6C3F1 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/w for a total of 64-65 exposures (over a 93-d period) with an interim sacrifice of rats after 20-21 exposures (over 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.

1, 0, 2 and 1 male mice exposed to 0, 100, 350 and 1000 ppm, respectively, spontaneously died during the experiment.

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.

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 106: Haematological parameters

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	Reticulocytes	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	Reticulocytes	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 (%)

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 107: Methemoglobinemia

Males					**Females**			
0	100	350	1000	Dose levels	0	10	350	1000
5	5	5	5	N	5	5	5	5
Immediately after the 64th (last) exposure (D92)								
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
4h after last exposure								
Not det.	Not det.	Not det.	7.4 ± 2.6	MetHb	Not det.	Not det.	Not det.	10.4 ± 2.9
19h 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

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 108: Clinical biochemistry parameters at interim kill

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/100ml); AP= alkaline phosphatase (mU/ml); P= phosphorus (mg/100ml); *p<0.05

Prior to the terminal kills (92 days), no effects were seen on SGPT, AP, 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, AP, glucose and phosphorus blood levels at terminal kill, in mice never bled before.

Table 109: 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/100ml); AP= alkaline phosphatase (mU/ml); P= phosphorus (mg/100ml); Ca= Calcium (mg/100ml)

Prior to the interim kill (30 days), no changes were found 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 were reported as well. 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, respectively) while 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, respectively). Furthermore, no changes in kidney relative weights were seen in females.

Prior to the terminal kills (92 days): No treatment-related effects on liver absolute and relative weights, were reported 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 110: Organ weights

	Males				**Females**			
Dose levels (ppm)	0	100	350	1000	0	100	350	1000
At interim kill								
N	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; rel= relative; abs= absolute

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 (probably the same animal) 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

Concerning histopathological findings, prior to the interim kill (30 days), hepatocellular vacuolization consistent with fat changes were noted in females exposed to 1000 ppm.
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 kills (92 days): 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 111: Histopathological modifications

	Males				**Females**			
Dose levels (ppm)	0	100	350	1000	0	100	350	1000
At interim sacrifice								
N animals	5	5	5	5	5	5	5	5
Liver: N 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: N tisssues examined:	5	0	0	4	-	-	-	-
Slight focal mononuclear aggregates	0	0	0	1	-	-	-	-
Prostate: N tissues examined	3	0	0	3	-	-	-	-
Slight focal mononuclear aggregates	2	0	0	3	-	-	-	-
Lungs: N 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: N 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: N 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: N 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: N 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: N 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: N 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: N 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: N tissues examined	5	0	0	5	5	0	0	5
Slight multifocal mononuclear aggregates	2	0	0	0	0	0	0	2
Tongue: N 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: N 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: N 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: N tissues examined	-	-	-	-	5	0	0	5

Primary benign teratoma, no metastasis	-	-	-	-	0	0	0	1
Cervix: N tissues examined	-	-	-	-	4	0	0	5
Very slight focal muscularis acute inflam.	-	-	-	-	0	0	0	1
Lacrimal gland: N 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; unilat.= unilateral; bilat.= bilateral

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 were 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 examination in mice dying spontaneously did not show effects 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 350 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

The LOAEC was determined at 350 ppm for males based on systemic effects on MetHb and liver after 13 weeks exposure.

This study is considered relevant for classification because the tested doses are in line with the guidance dose range relevant for classification (up to 350 ppm = 1 mg/L).

In a chronic inhalation study (Anonymous 35, 1986), rats were exposed during 2 years to either 0, 100, or 200 ppm nitroethane by inhalation. Mortality was relatively high in all dose group, without any dose-response relationship. Indeed, at least 50 % of the control group did not survive during the 2-year study (See Table 56)

No relevant effects were reported after clinical chemistry and haematology data assessment. Organ weights were not affected by the treatment. Methemoglobinemia was not examined. Concerning histopathology, no other effects than usual age-associated degenerative diseases and the endocrine target organ response to pituitary hyperplasia were observed and there were similar in controls and exposed animals.

Please refer to chapter 10.9.1.

For a 2-y study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 0.025 mg/L/d for Cat. 1 and 0.025 ≤ C ≤ 0.125 mg/L/d for Cat. 2, respectively. Therefore the data presented here are supportive information (Concentrations 0, 100, and 200 ppm corresponding approximatively to 0, 0.31 and 0.61 mg/L, respectively).

Case report

In a case study report (Hornfeldt and Rabe, 1994), a 20-month old boy ingested less than 30 mL of 100 % nitroethane from fingernail polish remover. In the Emergency Room, cyanosis and methemoglobinemia level of 39 % were reported. After an intravenous treatment with methylene blue, methemoglobin level decreased to 5.7 %. The boy fully recovered. No more data available.

In another case study report (Osterhoudt *et al.*, 1995), a 13-month old girl ingested fingernail polish remover first thought to be acetone-based. She weighted 10.2 kg, was healthy and under no medication. She first was brought to the emergency room without any symptom and sent home. Then 7 hours after ingestion, she came back and presented emesis and lethargy. The fingernail product was identified as 100 % nitroethane and maximum 90 mL was missing from the bottle. Cyanosis and tachypnea were observed. Oxygen (80 % supplement) was given but the girl remained in a cyanotic state. No cardiac symptom were reported; nor abdominal abnormalities. Methemoglobinemia was confirmed with blood analysis (Table 112). A rebound in methemoglobin increased its level up to 53 % 23 hours after ingestion.

Table 112: Methemoglobin levels, clinical symptoms and methylene blue dose

Time (hours)	% Methb	Clinical findings	Methylene Blue dose (mg/kg)
7	48	Emesis, lethargy, cyanosis	3.5
17	19	-	-
23	53	-	2
35	24	-	-
42	5.5	-	-
60	0.4	-	-

Total hemoglobin concentration was 10.7 g/dL, normal liver enzymes levels in serum and not deficient glucose-6-phosphate dehydrogenase were stated in the report.

Table 113: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Respiratory tract				
Range-finding of the 28-day repeated dose toxicity study in Rat Oral route 1-nitropropane Anonymous 38, 1996	No effect observed in respiratory tract (however nasal cavity not examined)	14 D	/	/
Short-term repeated dose toxicity study in Rat Oral route 1-nitropropane Anonymous 38, 1996	No effect observed in respiratory tract (however nasal cavity not examined)	28 D	/	/
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in	Degeneration and inflammation of the olf. epith. at 100 ppm corresp. approx. to	Male: min. 28 D Female: ± 45 D	Male: ± 0.12 mg/L Female: ± 0.18 mg/L	STOT RE Cat. 1 As ≤ 0.2 mg/L

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Rat Inhalation route 1-nitropropane Anonymous 37, 2003	0.369 mg/L			
16-day repeated dose toxicity study in Rat Inhalation route Nitromethane NTP, 1997	375 ppm corresp. approx. to 0.938 mg/L Degeneration olf. epith	16 D	± 0.17 mg/L	STOT RE Cat. 1 As ≤ 0.2 mg/L
16-day repeated dose toxicity study in Mouse Inhalation route Nitromethane NTP, 1997	375 ppm corresp. approx. to 0.938 mg/L Degeneration olf. epith.	16 D	± 0.17 mg/L	STOT RE Cat. 1 As ≤ 0.2 mg/L
13-week repeated dose toxicity study in Rat Inhalation route Nitromethane NTP, 1997	375 ppm corresp. approx.. to 0.938 mg/L Degeneration olf. epith. (+ hyaline droplets at 750 ppm)	13 W	0.398 mg/L	STOT RE Cat. 2 As 0.2 < C ≤ 1.0 mg/L
13-week repeated dose toxicity study in Mouse Inhalation route Nitromethane NTP, 1997	188 ppm corresp. approx.. to 0.47 mg/L Degeneration olf. epith. + hyaline droplets	13 W	0.47 mg/L	STOT RE Cat. 2 As 0.2 < C ≤ 1.0 mg/L
Sub-chronic repeated dose toxicity study in Rat Inhalation route Nitromethane Lewis *et al.*, 1977	No sign. effect in the repisratory tract (however, nasal cavity not examined microscopically)	13 W	/	/
Sub-chronic repeated dose toxicity study in Rabbit Inhalation route Nitromethane Lewis *et al.*, 1977	At 1-month: ≥100 ppm: effect observed in the lungs (focal area of hemorrhage, congestion of alveolar area) Nasal cavity not examined microscopically	At 1 month	± 0.1 mg/L	Indication of effect in the range to classify in Cat. 1 after 1 month of exposure
13-week repeated dose toxicity study in Rat Inhalation route	At interim sacrificed (± 1 month) Degeneration olf.	Interim sacrifice: ± 1 month	± 0.3 mg/L	STOT RE Cat. 1 after 1 month

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Nitroethane Anonymous 26, 1982	epith. + chronic inflammation already at 350 ppm (corresp. approx.. to 1.0 mg/L) Terminal sacrifice Moderate diffuse degeneration olf. epith. in all animals at 1000 ppm corresp. approx.. to 3.0 mg/L (slight at 350 ppm)	Terminal sacrifice: 92 D	3.0 mg/L	No classification
13-week repeated dose toxicity study in Mouse Inhalation route Nitroethane Anonymous 26, 1982	At interim sacrificed (± 1 month) Degeneration olf. epith. + inflammation + moderate glandular hyperplasia already at 350 ppm (corresp. approx.. to 1.0 mg/L) Terminal sacrifice Moderate degeneration olf. epith. + inflammation + moderate glandular hyperplasia already at 350 ppm (corresp. approx.. to 1 mg/L)	Interim sacrifice: ± 1 month Terminal sacrifice: 93 D	± 0.3 mg/L 1.0 mg/L	STOT RE Cat. 1 SOT RE Cat. 1 (borderline to Cat. 2)
2-year inhalation toxicity study in Rat Nitromethane NTP, 1997	No effect observed in respiratory tract	2 y	/	No classification
2-year inhalation toxicity study in Mouse Nitromethane NTP, 1997	≥ 188 ppm (cooresp. approx.. to 0.47 mg/L): sign increase degeneration olf. epith.	2 y	3.76 mg/L	No classification
Chronic inhalation toxicity study in Rat Nitroethane Anonymous 35, 1986	No effects observed	2 y	/	No classification
Blood				
Range-finding of the 28-day repeated dose toxicity study in Rat	150 mg/kg bw/d	14 D	25 mg/kg bw/d	STOT RE 2

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Oral route 1-nitropropane Anonymous 38, 1996				
Short-term repeated dose toxicity study in Rat Oral route 1-nitropropane Anonymous 38, 1996	100 mg/kg bw/d	28 D	33 mg/kg bw/d	STOT RE 2
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in Rat Inhalation route 1-nitropropane Anonymous 37, 2003	0.369 mg/L (slight decrease MetHb in M)	Male: min. 28 D Female: ± 45 D	0.123 mg/L	STOT RE 1 But only slight decrease MetHb Only very low dose tested
16-day repeated dose toxicity study in Rat Inhalation route Nitromethane NTP, 1997	Hematology not examined	16 D	/	/
16-day repeated dose toxicity study in Mouse Inhalation route Nitromethane NTP, 1997	Hematology not examined	16 D	/	/
13-week repeated dose toxicity study in Rat Inhalation route Nitromethane NTP, 1997	0.938 mg/L	13 W	0.938 mg/L	STOT RE 2
13-week repeated dose toxicity study in Mouse Inhalation route Nitromethane NTP, 1997	3.75 mg/L (extramedullary hematopoiesis in spleen)	13 W	3.75 mg/L	No classification But hematology not peformed
Sub-chronic repeated dose toxicity study in Rat Inhalation route Nitromethane	1.875 mg/L	13 W	1.875 mg/L	No classification

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Lewis *et al.*, 1977				
Sub-chronic repeated dose toxicity study in Rabbit Inhalation route Nitromethane Lewis *et al.*, 1977	1.875 mg/L (Hb reduced at 1 month)	1 month	0.625 mg/L	STOT RE 2
13-week repeated dose toxicity study in Rat Inhalation route Nitroethane Anonymous 26, 1982	0.3 mg/L	Terminal sacrifice: 92 D	0.3 mg/L	STOT RE 2
13-week repeated dose toxicity study in Mouse Inhalation route Nitroethane Anonymous 26, 1982	3.0 mg/L	Interim sacrifice: ± 1 month Terminal sacrifice: 93 D	3.0 mg/L	No classification
2-year inhalation toxicity study in Rat Nitromethane NTP, 1997	Hematology not examined	2 Y	/	/
2-year inhalation toxicity study in Mouse Nitromethane NTP, 1997	Hematology not examined	2 Y	/	/
Chronic inhalation toxicity study in Rat Nitroethane Anonymous 35, 1986	No effects observed However MetHb not examined	2 Y	/	/
Nervous system				
Range-finding of the 28-day repeated dose toxicity study in Rat Oral route	150 mg/kg bw/d	14 D	25 mg/kg bw/d	STOT RE 2

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
1-nitropropane Anonymous 38, 1996				
Short-term repeated dose toxicity study in Rat Oral route 1-nitropropane Anonymous 38, 1996	100 mg/kg bw/d	28 D	33 mg/kg bw/d	STOT RE 2
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in Rat Inhalation route 1-nitropropane Anonymous 37, 2003	0.369 mg/L in M	Male: min. 28 D Female: ± 45 D	0.123 mg/L	STOT RE 1
16-day repeated dose toxicity study in Rat Inhalation route Nitromethane NTP, 1997	0.938 mg/L	16 D	0.16 mg/L	STOT RE 1
16-day repeated dose toxicity study in Mouse Inhalation route Nitromethane NTP, 1997	3.75 mg/L	16 D	0.625 mg/L	STOT RE 2 (but only clinical signs observed)
13-week repeated dose toxicity study in Rat Inhalation route Nitromethane NTP, 1997	0.938 mg/L	13 W	0.938 mg/L	STOT RE 2
13-week repeated dose toxicity study in Mouse Inhalation route Nitromethane NTP, 1997	No effects observed	13 W	/	No classification
Sub-chronic repeated dose toxicity study in Rat Inhalation route Nitromethane Lewis *et al.*, 1977	No effects observed	13 W	/	No classification

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Sub-chronic repeated dose toxicity study in Rabbit Inhalation route Nitromethane Lewis *et al.*, 1977	No effects observed	At 1 month	/	No classification
13-week repeated dose toxicity study in Rat Inhalation route Nitroethane Anonymous 26, 1982	No effects observed	Interim sacrifice: ± 1 month Terminal sacrifice: 92 D	/	No classification
13-week repeated dose toxicity study in Mouse Inhalation route Nitroethane Anonymous 26, 1982	3.0 mg/L	Terminal sacrifice: 93 D	3.0 mg/L	No classification
2-year inhalation toxicity study in Rat Nitromethane NTP, 1997	No effects observed	2 Y	/	No classification
2-year inhalation toxicity study in Mouse Nitromethane NTP, 1997	No effects observed	2 Y	/	No classification
Chronic inhalation toxicity study in Rat Nitroethane Anonymous 35, 1986	No effects observed	2 Y	/	No classification

10.12.2 Comparison with the CLP criteria

Criteria for STOT RE 1	Criteria for STOT RE 2
"Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in category 1 for target	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target toxicity (repeat exposure) on the basis of observations

organ toxicity (repeat exposure) on the basis of:

- Reliable and good quality evidence from human cases or epidemiological studies; or
- Observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations."

"Classification in category 1 is applicable, when significant toxic effects observed in a 90-day repeated dose study conducted in experimental animals are seen to occur at or below the guidance value (C) as indicated in table 3.9.2"

Table 3.9.2

Route of exposure	Units	Guidance value
Oral (rat)	mg/kg bw/d	C≤10

from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations."

"Classification in category 2 is applicable, when significant toxic effects observed in a 90-day repeated dose study conducted in experimental animals are seen to occur within the guidance value range as indicated in table 3.9.3"

Table 3.9.3

Route of exposure	Units	Guidance value range
Oral (rat)	mg/kg bw/d	10 < C ≤ 100

Annex I of the CLP guidance: 3.9.2.7.3. *"Evidence from appropriate studies in experimental animals can furnish much more detail, in the form of clinical observations, haematology, clinical chemistry, and macroscopic and microscopic pathological examination, and this can often reveal hazards that may not be life-threatening but could indicate functional impairment. Consequently all available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:*

(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites.

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g., sight, hearing and sense of smell).

(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters.

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination.

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity.

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver).

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration."

- **Respiratory tract**

Subacute toxicity studies

Subacute toxicity studies were available for 1-nitropropane and nitromethane (See Table 114).

In the range-finding of the 28-day repeated dose toxicity (Anonymous 38, 1996) as well as in the 28-day repeated dose toxicity (Anonymous 38, 1996) performed with 1-nitropropane, no effects were observed in the respiratory tract after an exposure by oral route. However, histopathology of the nasal cavity was not performed. While in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Anonymous 37, 2003), 1-nitropropane was administered via inhalation to rats. In this study, degeneration of the olfactory epithelium was observed at the highest tested dose which is comprised in the range to classify in category 1. Same effects, observed at doses warranted a classification in category 1, were observed in the 16-day repeated dose toxicity study performed with nitromethane in rat and mouse (NTP, 1997).

Table 114: Summary data about respiratory tract in the subacute toxicity study

		Guidance value range for warranting classification	DS's conclusion
1-Nitropropane			
Range-finding of the 28-day repeated dose toxicity study Oral route Rat (SD) 3/sex/dose 0, 10, 50 150 and 250 mg/kg bw/d 14 D of exposure Anonymous 38, 1996	No effects observed in respiratory tract However nasal cavity not examined	Cat. 2: > 60 and ≤ 600 mg/kg bw/d Cat. 1: C ≤ 60 mg/kg bw/d	No classification based on the result but nasal cavity not examined microscopically
Short-term repeated dose toxicity study Oral route Rat (SD) 5/sex/dose 0, 10, 30 and 100 mg/kg bw/d (and 2 recovery groups: 0 and 100 mg/kg bw/d) 28 D of exposure (Recovery period: 14 D) Anonymous 38, 1996	No effects observed in respiratory tract However nasal cavity not examined	Cat. 2: > 30 and ≤ 300 mg/kg bw/d Cat. 1: C ≤ 30 mg/kg bw/d	No classification based on the result but nasal cavity not examined microscopically
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Inhalation route Rat (SD) 12/sex/dose 0, 25, 50 and 100 ppm (± 0, 0.092, 0.184 and 0.369 mg/L) Males: minimum 28 D	Degeneration of the olf. epith. (multifocal) in 7 F (5 VS and 2 S) and in 2 M (1 VS and 1 S) at 100 ppm (not observed in the other groups) Degeneration olf. epith. with inflammation (focal) in 2 F (VS) at 50 ppm and in 2 F (S) at 100 ppm Degeneration olf. epith. with inflammation (multifocal) in 2 F	For 28 D of exposure Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: C ≤ 0.6 mg/L/6 h/d For ± 45 D of exposure	Degeneration and inflammation observed at dose relevant to classify in Cat. 1 Only very low doses tested

Females: ± 45 D Anonymous 37, 2003	(S) at 100 ppm Chronic inflammation of epith (squamous cell, multifocal): VS in 1, 1, 1 and 2 F and S in 0, 0, 2 and 1 F	Cat. 2: : 0.4 < C ≤ 2 mg/L/6 h/d Cat. 1: C ≤ 0.4 mg/L/6 h/d	
Nitromethane			
16-day repeated dose toxicity study Inhalation route Rat (F344) 5/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	1500 ppm: Rapid breathing ≥ 375 ppm: sign. increased inc. of minimal to mild degeneration of the olfactory epithelium	Cat. 2: 1.2 < C ≤ 6 mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	Degeneration observed at doses within the range to classify in Cat. 1
16-day repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	1500 ppm: tachypnea in both sexes ≥ 375 ppm: sign. increased inc. of degeneration of the olfactory epithelium of the nose in all males and females (minimal severity in males and minimal to mild severity in females).	Cat. 2: 1.2 < C ≤ 6 mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	Degeneration observed at doses within the range to classify in Cat. 1
Nitroethane			
No subacute toxicity study available	/	/	/

Sub-chronic toxicity studies

Sub-chronic toxicity studies were available with nitromethane and nitroethane.

As the sub-acute toxicity studies, both substances affected the respiratory tract after a sub-chronic exposure. For nitromethane, the 2 studies performed in rat and mouse (NTP, 1997) exhibited a significant increased incidence of degeneration of the olfactive epithelium at dose which warrant a classification in category 2. Same effects were noted in the studies performed with nitroethane (Anonymous 26, 1982) and these effects were also observed at dose level which are within the range to classify in category 2.

Table 115: Summary data about respiratory tract in sub-chronic toxicity study

		Guidance value range for warranting classification	DS's conclusion
Nitromethane			
13-week repeated dose toxicity study Inhalation route Rat (F344) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938,	≥ 375 ppm: Degeneration of the olf. epith. in both sexes (in 0, /, 0, 9**, 10** and 10** M and in 0, 0, 1, 10**, 10** and 10** F) ≥ 750 ppm: Hyaline droplets olf. epith. (0, /, 0, 0, 1 and 8** M and 0, 0, 0, 0, 4* and 10** F)	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Sign increased inc. of degeneration olf. epith. at dose relevant to classify in Cat. 2

1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997			
13-week repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	≥ 375 ppm: Degeneration olf. epith in M (0, 0, 0, 10**, 10** and 10**) + Hyaline droplets olf. epith. (0, 0, 1, 10**, 10** and 10**) ≥ 188 ppm: Degeneration olf. epith in F (0, 0, 7**, 10**, 10** and 10**) + Hyaline droplets olf. epith. (0, 2, 9**, 10**, 10** and 10**)	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Increased inc. of degeneration olf. epith. at doses within the range to classify in Cat. 2
Sub-chronic repeated dose toxicity study Inhalation route Rat (SD) 50 M/dose 100 and 750 ppm (± 0.25 and 1.875 mg/L) 13 w of exposure Lewis *et al.*, 1977	No sign. increased incidence of effect in respiratory tract. However, nasal cavity not examined microscopically	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d	No classification
Sub-chronic repeated dose toxicity study Inhalation route Rabbit (NZW) 15 M/dose 100 and 750 ppm (± 0.25 and 1.875 mg/L) 13 w of exposure Lewis *et al.*, 1977	≥ 100 ppm: at the 1-month time point, modifications in the lungs as focal aeras of mild to severe hemorrhage and congestion of the alveolar area and duct walls. Interstitial edema of the alveolar and alveolar duct walls and some degree of alveolar wall necrosis seen in the area of hemorrhage and congestion.	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d For the 1-month time point: 0.6 < C ≤ 3 mg/L/6 h/d	Indication of respiratory effects (after 1 month) at dose to classify in Cat. 1
Nitroethane			
13-week repeated dose inhalation toxicity study Inhalation route Rat (F344) 15/sex/dose 0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L)	At interim sacrifice (5 animals/sex/group examined): ± 1 month Slight diffuse degeneration olf. epith. in 3 M at 350 ppm and in 5 M and 5 F at 1000 ppm Slight chronic active inflammation olf. epith in 1 F at 100 ppm, in 5 M and 1 F at 350 ppm and in 5 M and 5 F at 1000 ppm	For interim kill: 30-day Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill	Effects already observed at doses within the range to classify in Cat. 2

92 D Anonymous 26, 1982	At terminal kill At 1000 ppm: Moderate diffuse degeneration olf. epith in 5 M and 5 F (out of 5/sex tested) (Slight in 1 M and 2 F at 350 ppm) + slight diffuse chronic active inflammation olf. epith. in 4 M and 5 F (out of 5 tested/sex) (also in 2 F at 350 ppm)	(90-day) Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	
13-week repeated dose inhalation toxicity study Mouse (B6C3F1) 5/sex/dose 0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L) 93 D Anonymous 26, 1982	At interim sacrificed (5 animals/sex/group examined): ± 1 month Moderate olf. epith. degeneration + inflammation in 3 M and 4 F at 350 ppm and in 4 M and 5 F at 1000 ppm Moderate glandular hyperplasia olf. epith. in 2 M and 4 F at 350 ppm and in 4 M and 4 F at 1000 ppm At terminal sacrifice (5 animals/sex/group examined) Moderate olf. epith. degeneration + inflammation in 4 M and 5 F at 350 ppm and in 5 M and 5 F at 1000 ppm Moderate glandular hyperplasia olf. epith. in 4 M and 5 F at 350 ppm and in 4 M and 5 F at 1000 ppm	For interim kill: 30-day Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill (90-day) Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Effects already observed at doses within the range to classify in Cat. 2

Chronic toxicity studies

Three chronic repeated dose toxicity studies are available (2 with nitromethane and 1 with nitroethane).

As observed in Table 116, no effect was observed in 2 of these studies. While, in one of the studies performed with nitromethane, degeneration of the olfactive epithelium was observed however at dose which does not warrant a classification.

Table 116: Summary data about respiratory tract in chronic toxicity study

		Guidance value range for warranting classification	DS's conclusion
Nitromethane			
2-year repeated dose inhalation toxicity study Inhalation route Rats (Fischer F344/N) 0, 94, 188 and 375 ppm (± 0, 0.235, 0.47 and 0.94 mg/L) 2 y of exposure NTP, 1997	No effects	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	/
2-year repeated dose inhalation toxicity study Inhalation route Mouse (B6C3F1) 50/sex/dose 0, 188, 375 and 750 ppm (±	≥188 ppm: sign DR ↑ olf. epith. degeneration	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	Effect outside the range to classify in Cat. 2 However, effect observed at the lowest tested dose.

0, 0.47, 0.94 and 1.87 mg/L) 2 y of exposure NTP, 1997			
Nitroethane			
Chronic inhalation toxicity study Inhalation route Rat (Long-Evans) 40/sex/dose 0, 100 and 200 ppm (± 0.31 and 0.61 mg/L) Anonymous 35, 1986	No effect	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	No classification

Conclusion for respiratory tract:

The dossier submitter acknowledges that the results provided in the sub-acute toxicity studies support a classification as STOT RE category 1. The dossier submitter is of the opinion to rely on the 90-day toxicity study results which **support a classification as STOT RE 2 for respiratory tract** considering that:

- A 90-d study is more appropriate to compare with the Guidance proposed standard range to classify than with an extrapolated range from a 16-d study.
- Most effects on the respiratory system are reported only in NTP, 1997.
- The effects observed in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test are described as very slight and slight.
- And no information on the respiratory system is given in the available human data.

- **Nervous system**

Sub-acute toxicity studies

Sub-acute toxicity studies were available for 1-nitropropane and nitromethane.

As observed in Table 117, studies performed with 1-nitropropane showed nervous effects at doses which warrant a classification. For two of them, effects were noted at doses to classify in category 2. The third study revealed brain weight modification at dose warranted a classification. In this study, the tested doses were very low. Furthermore, the study performed in rat with nitromethane revealed degeneration of the sciatic nerve observed at dose warranting a classification in category 1.

Table 117: Summary data about nervous system in the subacute toxicity study

		Guidance value range for warranting classification	DS's conclusion
1-Nitropropane			
Range-finding of the 28-day repeated dose toxicity study	At 150 and 250 mg/kg bw/d: clinical signs such as ataxia, body tremors, loss of righting	Cat. 2: > 60 and ≤ 600 mg/kg bw/d Cat. 1: C ≤ 60	Clinical signs on nervous system at dose supproting Cat.

Oral route Rat (SD) 3/sex/dose 0, 10, 50 150 and 250 mg/kg bw/d 14 D of exposure Anonymous 38, 1996	reflex, lethargy At 250 mg/kg bw/d: all animals died during the study	mg/kg bw/d	2
Short-term repeated dose toxicity study Oral route Rat (SD) 5/sex/dose 0, 10, 30 and 100 mg/kg bw/d (and 2 recovery groups: 0 and 100 mg/kg bw/d) 28 D of exposure (Recovery period: 14 D) Anonymous 38, 1996	In M: Sign ↑ abs and rela brain weight at the highest dose In F: Sign ↑ abs brain weight at the mid and high doses	Cat. 2: > 30 and ≤ 300 mg/kg bw/d Cat. 1: C ≤ 30 mg/kg bw/d	Brain weight modified at dose supporting Cat 2
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Inhalation route Rat (SD) 12/sex/dose 0, 25, 50 and 100 ppm (± 0, 0.092, 0.184 and 0.369 mg/L) Males: minimum 28 D Females: ± 45 D Anonymous 37, 2003	In M: Sign ↑ rela brain weight at the highest dose	For 28 D of exposure Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: C ≤ 0.6 mg/L/6 h/d For ± 45 D of exposure Cat. 2: : 0.4 < C ≤ 2 mg/L/6 h/d Cat. 1: C ≤ 0.4 mg/L/6 h/d	Brain weight modified at dose supporting Cat 1 Only very low dose tested
Nitromethane			
16-day repeated dose toxicity study Inhalation route Rat (F344) 5/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	1500 ppm: hyperactivity at the beginning and hypoactivity and loss of coordination in hindlimbs at the end of the study ≥ 750 ppm: reduced myelin around sciatic nerve ≥ 375 ppm: sign. and DR increase inc. of sciatic nerve degeneration (in all animals at the 3 highest doses)	Cat. 2: 1.2 < C ≤ 6 mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	STOT RE Cat. 1 Sciatic nerve degeneration already observed at ≥ 0.938 mg/L
16-day repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L)	1500 ppm: reduced activity (sciatic nerve not examined)	Cat. 2: 1.2 < C ≤ 6 mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	STOT RE 2 (but only clinical signs)

NTP, 1997			
Nitroethane			
No subacute toxicity study available	/	/	/

Sub-chronic toxicity studies

As observed in one sub-acute toxicity study, degeneration of the sciatic nerve was observed in the 13-week repeated dose toxicity study performed with nitromethane on the rat. In this case, the effects observed are noted in the range to classify in category 2. The other sub-chronic toxicity studies did not demonstrate nervous system effects, however the sciatic nerve and other nerves were not examined in all the studies.

Table 118: Summary data on nervous system after sub-chronic exposure

		Guidance value range for warranting classification	DS's conclusion
Nitromethane			
13-week repeated dose toxicity study Inhalation route Rat (F344) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	1500 ppm: hindlimbs paralysis in all animals (starting from D 21) 750 ppm: hindlimbs paralysis in 1 M and 4 F (starting from D 63) ≥ 750 ppm: grip strength sign. reduced ≥ 375 ppm: sign. and DR increase inc. of sciatic nerve degeneration (in 5**, 10** and 10** M and in 8**, 10** and 10** F, resp. at 375, 750 and 1500 ppm) and spinal cord degeneration (in 9**, 10** and 10** M and in 2, 10** and 10** F, resp. at 375, 750 and 1500 ppm) + startle response amplitude ended to decrease	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	STOT RE Cat. 2 At 375 ppm (corresp. approx.. to 0.938 mg/L): sign. increase sciatic nerve and spinal cord degeneration + at the highest dose, hindlimbs paralysis observed after 21 D of exposure
13-week repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	No effects observed Neurobehavioral measurement not performed	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	No classification
Sub-chronic repeated dose toxicity study Inhalation route Rat (SD) 50 M/dose	No effects observed	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d	No classification

100 and 750 ppm (± 0.25 and 1.875 mg/L) 13 w of exposure Lewis *et al.*, 1977			
Sub-chronic repeated dose toxicity study Inhalation route Rabbit (NZW) 15 M/dose 100 and 750 ppm (± 0.25 and 1.875 mg/L) 13 w of exposure Lewis *et al.*, 1977	No effects observed	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d For the 1-month time point: 0.6 < C ≤ 3 mg/L/6 h/d	No classification
Nitroethane			
13-week repeated dose inhalation toxicity study Inhalation route Rat (F344) 15/sex/dose 0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L) 92 D Anonymous 26, 1982	No effects observed	For interim kill: 30-day Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill (90-day) Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	No classification
13-week repeated dose inhalation toxicity study Mouse (B6C3F1) 5/sex/dose 0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L) 93 D Anonymous 26, 1982	Abs and rela brain weight sign. ↓ at the highest dose (DR) No microscopic effects	For interim kill: 30-day Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill (90-day) Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	No classification

Chronic toxicity studies:

As observed in the table below, none studies demonsrated nervous effects after a chronic exposure.

Table 119: Summary data on nervous system after chronic exposure

		Guidance value range for warranting classification	DS's conclusion
Nitromethane			
2-year repeated dose inhalation toxicity study Inhalation route Rats (Fischer F344/N) 0, 94, 188 and 375 ppm (± 0, 0.235, 0.47 and 0.94 mg/L) 2 y of exposure NTP, 1997	No effects observed	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	No classification
2-year repeated dose inhalation toxicity study Inhalation route Mouse (B6C3F1) 50/sex/dose 0, 188, 375 and 750 ppm (± 0, 0.47, 0.94 and 1.87 mg/L) 2 y of exposure NTP, 1997	No effects observed	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	No classification
Nitroethane			
Chronic inhalation toxicity study Inhalation route Rat (Long-Evans) 40/sex/dose 0, 100 and 200 ppm (± 0.31 and 0.61 mg/L) Anonymous 35, 1986	No effects observed	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	No classification

Conclusion for nervous system:

Based on effects seen in the rat: degeneration of the sciatic nerve and the spinal cord starting from 375 ppm nitromethane in the 13-week inhalation repeated dose toxicity study (NTP, 1997), and supported by similar effects in the rat 16-day repeated dose toxicity study at the same dose level.

In the 13-week inhalation repeated dose toxicity study in rat, supportive neurotoxic effects were reported as hindlimbs paralysis and decreased hindlimb and forelimb grip strength at higher dose (1500 ppm nitromethane) and indicate that those effects are of concern. However, examination of the spinal cord and sciatic nerve did not reveal any effects in the 2-year inhalation study in the rat (NTP, 1997).

In the 28-day oral repeated dose toxicity study performed with 1-nitropropane in rat (Anonymous 38, 1996), a statistically significantly increased brain weights in females at 30 mg/kg bw/d was observed. At 100 mg/kg bw/d, this effect was reported in both sexes.

Human data demonstrated severe axonal neuropathy diagnosed in 2 workers after exposure to nitromethane by inhalation (Page *et al.*, 2001). Co-exposure to other chemicals cannot be excluded but according to the authors, nitromethane is likely to be the cause of the symptoms.

The dossier submitter acknowledges that the results provided in the 16-day inhalation repeated dose toxicity study support a classification as STOT RE category 1. The dossier submitter is of the opinion to rely on the 90-day toxicity study results which support a **classification as STOT RE 2 for nervous system** because:

- Human data is available, but only on 2 workers.
- Neurotoxic effects were seen in different studies (NTP, 1997 and Anonymous 38, 1996).
- Neurotoxicity was not examined in the mouse.
- A 90-d study is more appropriate to compare with the Guidance proposed standard range to classify than with an extrapolated range from a 16-day repeated dose toxicity study

➢ **Blood**

Sub-acute toxicity studies:

After a sub-acute exposure to 1-nitropropane, hematological effects were showed in different studies at doses warranting a classification in Category 2.

Table 120: Summary data on hematological effects after sub-acute exposure

		Guidance value range for warranting classification	DS's conclusion
1-Nitropropane			
Range-finding of the 28-day repeated dose toxicity study Oral route Rat (SD) 3/sex/dose 0, 10, 50 150 and 250 mg/kg bw/d 14 D of exposure Anonymous 38, 1996	At 150 and 250 mg/kg bw/d: clinical signs such as pallor of extremities, lethargy + pale kidneys Only at 250 mg/kg bw/d: pale liver and adrenals At 250 mg/kg bw/d: all animals died during the study	Cat. 2: > 60 and ≤ 600 mg/kg bw/d Cat. 1: C ≤ 60 mg/kg bw/d	Clinical signs at dose supporting Cat. 2
Short-term repeated dose toxicity study Oral route Rat (SD) 5/sex/dose 0, 10, 30 and 100 mg/kg bw/d (and 2 recovery groups: 0 and 100 mg/kg bw/d) 28 D of exposure (Recovery period: 14 D) Anonymous 38, 1996	In F: at 100 mg/kg bw/d: Sign. and DR ↓ of Hb, Ht and RBC (also observed in recovery group) MetHb: ↑ DR (not sign.)	Cat. 2: > 30 and ≤ 300 mg/kg bw/d Cat. 1: C ≤ 30 mg/kg bw/d	Sign. and DR hematological effects supporting Cat. 2
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Inhalation route Rat (SD) 12/sex/dose 0, 25, 50 and 100 ppm (± 0, 0.092, 0.184 and 0.369 mg/L)	MetHb: 1.7, 1.6, 1.6 and 1.5 % in M and 1.0, 1.0, 1.5 and 1.0 % in F	For 28 D of exposure Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: C ≤ 0.6 mg/L/6 h/d	Slight decrease MetHb in M Only very low doses tested

Males: minimum 28 D Females: ± 45 D Anonymous 37, 2003		For ± 45 D of exposure Cat. 2: : 0.4 < C ≤ 2 mg/L/6 h/d Cat. 1: C ≤ 0.4 mg/L/6 h/d	
Nitromethane			
16-day repeated dose toxicity study Inhalation route Rat (F344) 5/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	Not examined	Cat. 2: 1.2 < C ≤ 6 mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	/
16-day repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	Not examined	Cat. 2: 1.2 < C ≤ 6 mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	/
Nitroethane			
No subacute toxicity study available	/	/	/

Sub-chronic exposure:

Table 121: Summary data on hematological effects observed after a sub-chonic exposure

		Guidance value range for warranting classification	DS's conclusion
Nitromethane			
13-week repeated dose toxicity study Inhalation route Rat (F344) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	Concentration-dependent, microcytic responsive anemia Characterized by mild to moderate decreases in Ht and Hb values and minimal to moderate decreases in mean cell volume at all time points at ≥ 375 ppm Platelets count midly to markedly increased in all treated group MetHb increased in M at ≥ 375 ppm and in F at 750 ppm and 1500 ppm	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Effects observed at doses supporting Cat. 2
13-week repeated dose toxicity study Inhalation route Mouse (B6C3F1)	Minimal extramedullary hematopoiesis in spleen at 1500 pm (in 0, 1, 0, 1, 2 and 10 M and in 0, 0, 0, 2, 3 and 9** F, resp. at 0, 94, 188, 375, 750 and 1500 ppm)	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d; dose of 188 and 375 ppm relevant for classification	No effects observed at doses warranting a classification However, hematological

10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	Hematological examination not performed	Cat. 1: ≤ 0.2 mg/L/6 h/d	examination not performed
Sub-chronic repeated dose toxicity study Inhalation route Rat (SD) 50 M/dose 100 and 750 ppm (± 0.25 and 1.875 mg/L) 13 w of exposure Lewis *et al.*, 1977	750 ppm: sign. Decrease in Ht and Hb MetHb not sign. modified	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d	No classification
Sub-chronic repeated dose toxicity study Inhalation route Rabbit (NZW) 15 M/dose 100 and 750 ppm (± 0.25 and 1.875 mg/L) 13 w of exposure Lewis *et al.*, 1977	Hb reduced at 1 month at the highest dose (no info for 100 ppm)	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d For the 1-month time point: 0.6 < C ≤ 3 mg/L/6 h/d	STOT RE 2
Nitroethane			
13-week repeated dose inhalation toxicity study Inhalation route Rat (F344) 15/sex/dose 0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L) 92 D Anonymous 26, 1982	1000 ppm: sign. increase in methemoglobin levels (associated with cyaniosis), in reticulocytes and Heinz bodies in blood associated with splenic congestion and extramedullary hematopoiesis Increase inc. of spleen darkness in all animals at 1000 ppm and in 9 M at 350 ppm Increase inc. of spleen congestion (in all M at ≥100 ppm and in 5, 4 and 5 F, resp. at 100, 350 and 1000 ppm) + extramedullary hematopoiesis (in all M at ≥100 ppm and in 1, 2 and 1 F, resp. at 100, 350 and 1000 ppm)	For interim kill: 30-day Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill (90-day) Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Supporting Cat. 2 based on microscopic effects
13-week repeated dose inhalation toxicity study Mouse (B6C3F1) 5/sex/dose 0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L)	MetHb sign increase at 1000 ppm in F Heinz bodies sign. ↑ in both sexes at 1000 ppm (tend to ↑ at low and mid doses) No splenic microscopic effects observed	For interim kill: 30-day Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill (90-day)	No classification

93 D Anonymous 26, 1982		Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	

Chronic exposure:

Table 122: Summary data on hematological effects after chronic exposure

		Guidance value range for warranting classification	DS's conclusion
Nitromethane			
2-year repeated dose inhalation toxicity study Inhalation route Rats (Fischer F344/N) 0, 94, 188 and 375 ppm (± 0, 0.235, 0.47 and 0.94 mg/L) 2 y of exposure NTP, 1997	Hematological examination not performed	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	/
2-year repeated dose inhalation toxicity study Inhalation route Mouse (B6C3F1) 50/sex/dose 0, 188, 375 and 750 ppm (± 0, 0.47, 0.94 and 1.87 mg/L) 2 y of exposure NTP, 1997	Hematological examination not performed	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	/
Nitroethane			
Long term inhalation toxicity study Inhalation route Rat (Long-Evans) 40/sex/dose 0, 100 and 200 ppm (± 0.31 and 0.61 mg/L) Anonymous 35, 1986	No effects observed However MetHb not examined	Cat. 2: 0.15 < C ≤ 0.75 mg/L/6 h/d Cat. 1: C ≤ 0.15 mg/L/6 h/d	/

Conclusion for blood:

Based on lower hemoglobin, hematocrit values and erythrocyte count, and a higher clotting time observed in the oral 28-day oral repeated dose toxicity study (Anonymous 38, 1996) at 100 mg/kg bw/d of 1-nitropropane, as well as effects on the methemoglobin seen in female rats exposed to 100 mg/kg bw/d 1-nitropropane, **a classification as STOT RE 2 for blood** is supported. Furthermore, effects on the methemoglobin were observed in both sexes in a dose-dependent way in rats exposed by inhalation to nitromethane for 13-week inhalation repeated dose toxicity study (NTP, 1997).

The NTP paper describes the effects as "*exposure to nitromethane caused an exposure concentration-dependent, microcytic, responsive anemia in rats. The anemia was characterized by mild to moderate*

decreases in hematocrit values and hemoglobin concentrations, and the microcytosis was evidenced by minimal to moderate decreases in mean cell volume.

- Conclusion

Degeneration of the olfactive epithelium, hematological effects and nervous system effects were considered treatment-related and adverse at relevant doses for classification for STOT RE, in category 2. In conclusion, a classification as **STOT RE Cat. 2** is proposed.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the available results, a classification as **STOT RE 2; H373 (May cause damage to organs through prolonged or repeated exposure) (blood, respiratory tract and nervous system)** is proposed.

10.13 Aspiration hazard

Not evaluated in this CLH dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this CLH dossier.

13 ADDITIONAL LABELLING

NA

14 ABBREVIATIONS

*: $p<0.05$

**: $p<0.01$

***: $p<0.001$

1-NP: 1-nitropropne

2-NP: 2-nitropropane

5 HIAA: 5-hydroxyindolacetic acid

Abs: absolute

ADME: Absorption, Distribution, Metabolism, and Excretion

ALP: alkaline phosphatase

ALT: alanine aminotransferase

Alv.: alveolar

Approx.: approximetaly

AST: aspartate aminotransferase

ATE: acute toxicity estimate

Avg.: average

B. or Bilat.: bilateral

BILI: bilirubin

Bronch.: bronchiolar

BUN: Blood urea nitrogen

BW: body weight

BWG: body weight gain

Cat.: category

CE: cloning efficiency

CHL: Chinese hamster lung

CHO: Chinese hamster ovary

Chrom.: chromosome

CMC: carboxymethylcellulose

Conc.: concentration

Corresp.: corresponding

CP: cyclophosphamide

CT: clotting time

D or d: day

DMSO: dimethylsulphoxide

DNA: Desoxyribo Nucleic Acid

DR: Dose-related

DS: dossier submitter

E. Coli: Escherichia coli

E.C.L. : estrous cycle length

ELISA : Enzyme-linked immunosorbent assay

Epith.: epithelium

F: female

FBW: final body weight

Flam.: flammable

g: gram

GD: gestational day

GLP: good laboratory practice

Gp: Group

GV: guidance value

H: hour

Hb: hemoglobin

HCD: historical control data

Hg: mercury

HGPRT: hypoxanthine-guanine phosphoribosyltransferase

Ht: hematocrit

IC95: interval confidence of 95 %

Impl.: implantation

Inc.: incidence

Inflam.: inflammation

IP: intraperitoneal

K: potassium

L.: left

LC100: lethal concentration 100%

LC50: lethal concentration 50%

LD50: lethal dose 50%

Liq.: liquid

LOAEC: low adverse effect concentration

LOAEL: low observed adverse effect level

Lymph: lymphocyte

M: male

Max: maximum

MCV: mean cell volume

Met. act.: metabolic activation

MetHb: methemoglobin

MHPG: 3-Methoxy-4-hydroxyphenylglycol

Min.: minimum

MMC: mitomycin C

MN: micronuclei

MNBC: miconucleated binucleated cells

Multifoc.: multifocal

Nb or N or No: number

NA: not applicable

NC: negative control

NCE: normochromatic erythrocytes

ND: not determined

NE: nitroethane

Neg.: negative

NM: nitromethane

NOAEC: no observed adverse effect concentration

NOAEL: no observed adverse effect level

NOEC: no effect concentration

Nucl.: nucleated

NZW: New Zealand White

O.E.: olfactory epitheliul

OCT: ornithine carbamyl transferase

Olf.: olfactory

PC: positive control

PCE: polychromatic erythrocytes

PCV: Pack cell volume

Plt: platelet

PND: post-natal day

Pos: positive

Prot: protein

Pt: prothrombin

R.E.: respiratory epithelium

RBC: red blood cell

RCS: relative cell survival

Rel: relative

Repr.: reproductive toxicity

Resp.: respectively

Resp. epith: respiratory epithelium

RPE: relative plating efficiency

S.: slight

S. Typh: Salmonella typhimurium

SCE: sister chromatid exchange

SD: Sprague-Dawley

SDH: serine dehydratase

SEM: standard error of mean

SHE: Syrian hamster embryo

Signif. or sign.: significant(-ly)

St. Dev.: standard deviation

STOT RE: Specific Target Organ Toxicity – Repeated Dose

STOT SE: Specific Target Organ Toxicity – Single Exposure

T3: Triiodothyronine

T4: Thyroxine

TCA cycle: Tricarboxilic acid cycle

TG: test guideline

Tot.: total

Tox.: toxicity

V.S.: very slight

WBC: white blood cell

Wng: warning

Y: year

15 ANNEXES

Confidential annex to CLH report

Annex I to CLH report

16 REFERENCES

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