

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: Nitromethane

EC Number: 200-876-6
CAS Number: 75-52-5
Index Number: 609-036-00-7

Contact details for dossier submitter:

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

CONTENTS


1	IDENTITY OF THE SUBSTANCE	1
1.1	NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE.....	1
1.2	COMPOSITION OF THE SUBSTANCE.....	1
2	PROPOSED HARMONISED CLASSIFICATION AND LABELLING	3
2.1	PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA.....	3
3	HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING	4
4	JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL	5
5	IDENTIFIED USES	5
6	DATA SOURCES	5
7	PHYSICOCHEMICAL PROPERTIES	6
8	EVALUATION OF PHYSICAL HAZARDS	13
9	TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)	13
9.1	SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S).....	13
10	EVALUATION OF HEALTH HAZARDS	14
10.1	ACUTE TOXICITY - ORAL ROUTE.....	14
10.1.1	<i>Short summary and overall relevance of the provided information on acute oral toxicity</i>	15
10.1.2	<i>Comparison with the CLP criteria</i>	18
10.1.3	<i>Conclusion on classification and labelling for acute oral toxicity</i>	18
10.2	ACUTE TOXICITY - DERMAL ROUTE.....	18
10.3	ACUTE TOXICITY - INHALATION ROUTE.....	18
10.3.1	<i>Short summary and overall relevance of the provided information on acute inhalation toxicity</i>	20
10.3.2	<i>Comparison with the CLP criteria</i>	23
10.3.3	<i>Conclusion on classification and labelling for acute inhalation toxicity</i>	23
10.4	SKIN CORROSION/IRRITATION.....	23
10.5	SERIOUS EYE DAMAGE/EYE IRRITATION.....	23
10.6	RESPIRATORY SENSITISATION.....	24
10.7	SKIN SENSITISATION.....	24
10.8	GERM CELL MUTAGENICITY.....	25
10.8.1	<i>Short summary and overall relevance of the provided information on germ cell mutagenicity</i>	34
10.8.2	<i>Comparison with the CLP criteria</i>	48
10.8.3	<i>Conclusion on classification and labelling for germ cell mutagenicity</i>	51
10.9	CARCINOGENICITY.....	52
10.9.1	<i>Short summary and overall relevance of the provided information on carcinogenicity</i>	58
10.9.2	<i>Comparison with the CLP criteria</i>	74
10.9.3	<i>Conclusion on classification and labelling for carcinogenicity</i>	76
10.10	REPRODUCTIVE TOXICITY.....	77
10.10.1	<i>Adverse effects on sexual function and fertility</i>	77
10.10.2	<i>Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility</i>	81
10.10.3	<i>Comparison with the CLP criteria</i>	89
10.10.4	<i>Adverse effects on development</i>	92
10.10.5	<i>Short summary and overall relevance of the provided information on adverse effects on development</i> 95	
10.10.6	<i>Comparison with the CLP criteria</i>	100
10.10.7	<i>Adverse effects on or via lactation</i>	103

10.10.8	<i>Short summary and overall relevance of the provided information on effects on or via lactation</i>	103
10.10.9	<i>Comparison with the CLP criteria</i>	104
10.10.10	<i>Conclusion on classification and labelling for reproductive toxicity</i>	104
10.11	SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE	104
10.12	SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE	106
10.12.1	<i>Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure</i>	120
10.12.2	<i>Comparison with the CLP criteria</i>	150
10.12.3	<i>Conclusion on classification and labelling for STOT RE</i>	164
10.13	ASPIRATION HAZARD	164
11	EVALUATION OF ENVIRONMENTAL HAZARDS	164
12	EVALUATION OF ADDITIONAL HAZARDS	164
13	ADDITIONAL LABELLING	165
14	ABBREVIATIONS	165
15	ANNEXES	168
16	REFERENCES	168

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)	Nitromethane
Other names (usual name, trade name, abbreviation)	Nitrometano Nitromethan
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	200-876-6
EC name (if available and appropriate)	Nitromethane
CAS number (if available)	75-52-5
Other identity code (if available)	/
Molecular formula	CH ₃ NO ₂
Structural formula	
SMILES notation (if available)	C[N+](=O)[O-]
Molecular weight or molecular weight range	61.0401 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)	≥ 99.0 % (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 (CLP)	in Table 3.1	Current classification and self-labelling (CLP)
Nitromethane EC n° 200-876-6	≥ 99.0 – 100 % (w/w)	Flam. Liq. 3, H226 Acute Tox. 4*, H302		Flam. Liq. 3, H226 Acute Tox. 4, H302

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

	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	609-036-00-7	nitromethane	200-876-6	75-52-5	Flam. Liq. 3 Acute Tox. 4*	H226 H302	GHS02 GHS07 Wng	H226 H302			
Dossier submitters proposal	609-036-00-7	nitromethane	200-876-6	75-52-5	Retain: Flam. Liq. 3 Modify: Acute Tox. 4 Add: Acute Tox. 3 Carc. 1B Repr. 1B STOT RE 2	Retain: H226 H302 Add: H331 H350 H360Df H373 (blood, respiratory tract and nervous system)	Retain: GHS02 GHS07 Remove: Wng Add: GHS08 Modify: Dgr	Retain: H302 Add: H331 H350 H360Df H373 (blood, respiratory tract and nervous system)		Add: ATE (oral) = 1450 mg/kg bw ATE (inhalation) = 5.50 mg/L (vapour)	
Resulting Annex VI entry if agreed by RAC and COM	609-036-00-7	nitromethane	200-876-6	75-52-5	Flam. Liq. 3 Acute Tox. 4 Acute Tox. 3 Carc. 1B Repr. 1B STOT RE 2	H226 H302 H331 H350 H360Df H373 (blood, respiratory tract and nervous system)	GHS02 GHS07 GHS08 Dgr	H226 H302 H331 H350 H360Df H373 (blood, respiratory tract and nervous system)		ATE (oral) = 1450 mg/kg bw 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	Hazard class not assessed in this dossier	No
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
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

Nitromethane is a chemical substance which is registered under REACH (1907/2006/EC). The substance is listed in annex VI of CLP (609-036-00-7) with the following classification:

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

Several self classifications are listed in the C&L inventory (consulted on the 28-11-2023): 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, H332

Acute Tox. 3, H331

Carc. 2, H351

Carc. 2, H351 (inhalation)

Repr. 2, H361

Repr. 2, H361 (fetotoxicity)

Eye Irrit. 2, H319

STOT SE 2, H371 (respiratory system...) (inhalation)

STOT RE 2, H373 (blood, thyroid,...)

Aquatic Chronic 3, H412

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

- * A classification is proposed for the endpoints reproductive toxicity and carcinogenicity. The substance is already self-classified as a reprotoxicant (Repr. 2, H361) and a carcinogen (Carc. 2, H351). Furthermore, new data is available (development/teratogenicity study with nitromethane).

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

This substance is used in the following products: pH regulators, water treatment products and laboratory chemicals, air care products, fuels, cosmetics and personal care products.

6 DATA SOURCES

Registration dossier (last consultation by the DS: September 2023

<https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/11417>)

C&L inventory: last consulted by the DS: 28-11-2023

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	Clear, colorless liquid	NTP, Technical Report on the Toxicology and Carcinogenesis Studies of Nitromethane in Fischer 344 rats and B6C3F1 Mice (inhalation studies), 1997	Reliability 1 (reliable without restriction) GLP
Melting/freezing point	-28.4 °C	Lide Dr., Handbook of Chemistry and Physics, 2003	Reliability 2 (reliable with restrictions) Data from handbook or collection of data
	-29.0 °C	Budavari, S., The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals, 1989	Reliability 2 (reliable with restrictions) Data from handbook or collection of data
	-28.6 °C	Toopa E.E., Chemical Research Memorandum Report No. 2082, 1955	Reliability 2 (reliable with restrictions) Data obtained from a standard literature reference on physical properties
Boiling point	101.2 °C at 760 mmHg	Anonymous 13, 1955	Reliability 2 (reliable with restrictions) Non-GLP, non-guideline Ebulliometer
	101.1 °C at 1 atm	Lide Dr., Handbook of Chemistry and Physics, 2000	Reliability 2 (reliable with restrictions) Data obtained from a peer reviewed handbook, non-guideline
Relative density	1.138 g/mL at 20.0 °C 1.131 g/mL at 25.0 °C 1.124 g/mL at 30.0 °C	Anonymous 13, 1955	Reliability 2 (reliable with restrictions) Non-GLP, Non-guideline Pycnometer method
	1.137 g/cm ³ at 20.0 °C	Lide Dr., Handbook of Chemistry and Physics, 2000	Reliability 2 (reliable with restrictions) Data obtained from a peer reviewed handbook
Vapour pressure	36.65 mmHg at 25.0 °C (Antoine equation) 74.76 mmHg at 40.0 °C (Antoine equation)	Anonymous 13, 1955	Reliability 2 (reliable with restrictions) Non-GLP, non-guideline Ebulliometer
	35.8 mmHg at 25.0 °C	Daubert and Danner, Physical and thermodynamic Properties of Pure Chemicals Data Compilation, 1989	Reliability 2 (reliable with restrictions) Non-guideline Data obtained from a peer reviewed handbook

CLH REPORT FOR NITROMETHANE

Property	Value	Reference	Comment (e.g. measured or estimated)
Surface tension	7.36 mN/m at 21.0 °C	Anonymous 14, 2011	Reliability 1 (reliable without restriction) GLP EU A.5 (Surface Tension) OECD TG 115 (Surface Tension of Aqueous Solutions) Ring method
Water solubility	104.5 g/L at 25.0 °C 10.5 % by wt at 20.0 °C 11.1 % by wt at 25.0 °C 19.3 % by wt at 70.0 °C	Anonymous 13, 1955	Reliability 2 (reliable with restrictions) Non-GLP, non-guideline
Partition coefficient n-octanol/water	Log Kow= 0.574 at 21.8 °C, pH 7	Anonymous 14, 2011	Reliability 1 (reliable without restriction) EU A.8 (Partition coefficient) Shake flask method to flask method
Flash point	36.0 °C at 101.5 kPa	Anonymous 14, 2011	Reliability 1 (reliable without restriction) GLP EU A.9 (Flash point) Closed cup
	35.0 °C at 760 mmHg	National fire protection association, Fire Protection Guide to Hazardous Materials, 2002	Reliability 2 (reliable with restrictions) Equivalent or similar to ISO 1523 (Determination of flash point - Closed cup equilibrium method) Data from handbook or collection of data with peer review
	35.0 °C at 760 mmHg	IPCS Inchem, 1997	Reliability 2 (reliable with restrictions) Closed cup Data from handbook or collection of data with peer review
	35.0 °C at 760 mmHg	Chemiekaarten, 12 ed, 1997	Reliability 2 (reliable with restrictions) Data from handbook or collection of data with peer review
Flammability	Substance is a liquid	/	/
Explosive properties	Non-explosive	Anonymous 14, 2011	Reliability 1 (reliable

Property	Value	Reference	Comment (e.g. measured or estimated)
			without restriction) GLP EU A.14 (Explosive properties)
	Explosive decomposition begins at 315.0 °C. May be detonated by nearby explosions.	National fire protection association , Fire Protection Guide to Hazardous Materials. 13 ed., 2002	Reliability 2 (reliable with restrictions) Data from handbook or collection of data with peer review
	Potentially very explosive, shock caused by sudden application of gas pressure, or sudden forced flow through restrictions, could detonate the liquid.	Bretherick L., Handbook of Reactive Chemical Hazards, 7th ed., 2007	Reliability 2 (reliable with restrictions) Data from handbook or collection of data with peer review
Self-ignition temperature	430 °C at $\geq 99.98 - \leq 101.91$ kPa	Anonymous 14, 2011	Reliability 1 (reliable without restriction) GLP EU A.15 (Auto-Ignition Temperature (Liquids and Gases))
	418 °C at 760 mmHg	National fire protection association , Fire Protection Guide to Hazardous Materials. 13 ed., 2002	Reliability 2 (reliable with restrictions) Data from handbook or collection of data with peer review
	417 °C at 760 mmHg	IPCS Inchem, 1997	Reliability 2 (reliable with restrictions) Data from handbook or collection of data with peer review
	415 °C at 760 mmHg	Chemiekaarten, 12 ed, 1997	Reliability 2 (reliable with restrictions) Data from handbook or collection of data with peer review
Oxidising properties	The nitroalkanes are mild oxidants under ordinary conditions, but precautions should be taken when they are subjected to high temperatures and pressures, since violent reactions may occur.	Bretherick L., Handbook of reactive chemical hazards, 4 th Ed., 1990	/
Granulometry	Substance is a liquid	/	/
Stability in organic solvents and identity of relevant degradation	/	/	/

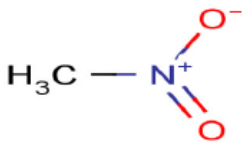
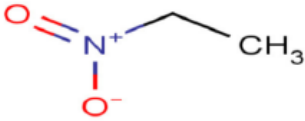
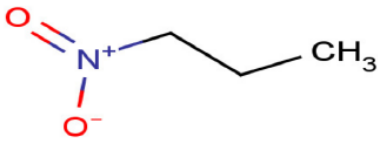
Property	Value	Reference	Comment (e.g. measured or estimated)
products			
Dissociation constant	10.20 ± 0.10	Anonymous 14, 2011	Reliability 2 (reliable with restrictions) QSAR
Viscosity	0.647 mPa s at 20 °C (dynamic viscosity)	Anonymous 13, 1955	Reliability 2 (reliable with restrictions) Non-guideline

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.*”

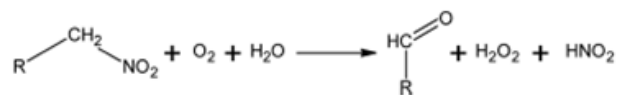
All three nitroalkanes are straight alkyl chain with similar molecular weights and only one single common functional group (Table 7). The only structural difference between nitromethane and nitroethane is a one carbon addition to the alkyl group. Further analogues differ in the length of the alkyl group 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

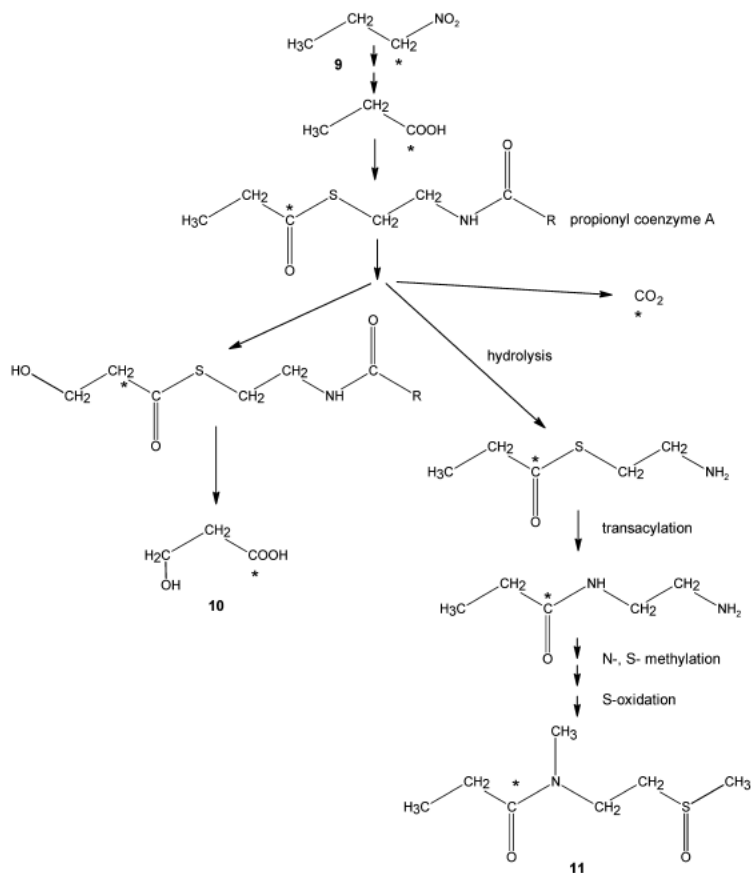
Substances:	N° CAS:	Molecular weight:
Nitromethane	75-52-5	61.04 g/mol
Nitroethane	79-24-3	75.07 g/mol
1-Nitropropane	108-03-2	89.09 g/mol
Structures:		
Nitromethane	Nitroethane	1-Nitropropane
		
<i>Physical state</i>		
Liquid	Liquid	Liquid

<i>Melting point (°C)</i>		
-28.4 °C	-89.5 °C	-104 °C
<i>Boiling point (°C)</i>		
101.2 °C at 1013 hPa	114 °C at 1013 hPa	131.1 °C at 1013 hPa
<i>Density</i>		
1.1322 g/cm ³ at 25 °C	1.0448 g/cm ³ at 25 °C	0.9934 g/cm ³ at 25 °C
<i>Vapour pressure (hPa)</i>		
37.1 hPa at 20 °C	27.7 hPa at 25 °C (estimated)	13 hPa at 25 °C (estimated)
<i>Water solubility (g/L at 20 °C)</i>		
111000 mg/L at 20 °C	45000 mg/L at 20 °C	15000 mg/L at 25 °C
<i>Partition coefficient n-octanol/water (log value)</i>		
-0.33	0.18	0.79
<i>Henry's law constant</i>		
2.1 Pa*m ³ /mol (estimated)	4.7 Pa*m ³ /mol	2.1 Pa*m ³ /mol (estimated)

Scheme 3. Action of Nitroalkane Oxygenase on a Primary Nitroalkane



Scheme 5. Biotransformation of 1-Nitropropane in Rats and Chimpanzees^a



^aAsterisks indicate the location of radiocarbon label (adapted from ref 46).

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)

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

In a non-guideline *in vitro* toxicokinetics study (Sakurai *et al.*, 1980), the liver microsomal metabolism of nitromethane and other aliphatic nitro compounds was assessed. First, rats were pretreated with either phenobarbital (80 mg/kg bw/d, during 3 days) or 3-methylcholanthrene (20 mg/kg bw/d, during 2 days) then sacrificed and the microsomal fraction was prepared. Nitromethane was then added at once to the *in vitro* system as 1 M methanolic solutions (50 mM nitromethane). Then, the release of metabolization products from nitromethane (nitrite and formaldehyde) was examined after 2, 4, 6 and 8 minutes. Findings showed the formation of a cytochrome P450-NO complex, the catalization by oxidized rat microsomes of the formaldehyde production in a not NADPH-dependent reaction and finally nitromethane was metabolized by the cytochrome P450 into formaldehyde and nitrate (1:1) in the presence of NADPH and dioxygen.

In a GLP non-guideline dermal absorption study (Anonymous 15, 1990), a single dermal dose (300 µL ether/ethanol solution containing 5.5 % ¹⁴C nitromethane) was applied on the intact skin by means of a disposable plastic syringe equipped with a feeding needle. Thereafter, the test site was covered with an occlusive plastic foil patch and taped air tight over the test site. Twelve hours after dose application the patch was removed and the skin was wiped with soap and acetone swabs to remove remaining test material. The swab and the patch were extracted with acetone and ethanol, respectively.

The extracts were assayed for radioactivity. Blood (i.e., plasma and erythrocytes separately), urine and feces samples were collected at several time points 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.

In the 72 hours after application, excretion of the test material was estimated to be 15.39 µg nitromethane (± 0.082 % of the total dose), 74.3 % of which was found in the urine. 48 hours after exposure, 90.4 % of the total urine radioactivity was excreted. 25.7 % of the total excreted radioactivity was observed in the feces.

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

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

72 hours after exposure, the skin was excised and samples contained 3.49 µg nitromethane (± 0.018 % of the total dose). No nitromethane was detected in subcutaneous fat. Also, 12 hours after exposure, 2.02 µg nitromethane (± 0,011 % of the total dose) was obtained by wiping the skin with soap/water and acetone swabs. Also, 0.76 µg nitromethane (± 0.004 % of the total dose) was recovered in the occlusive patch. The high loss of nitromethane (18.84 mg, ± 99.88 % of the total dose) was probably thought to be due to the volatility of the test material and thus evaporation (despite the patch) from the test site. Plus, the registrant claimed that exhaled radioactivity as nitromethane or volatile metabolites could not be detected under the test conditions.

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

This 14C test does not allow to identify if it was nitromethane or a metabolite that was detected.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Table 8: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD ₅₀	Reference
<p>Acute oral toxicity study</p> <p>No guideline</p> <p>Not GLP compliant</p> <p>Reliability 2 (according to the registration dossier, study was not made available to the DS)</p>	<p>Rats (SD)</p> <p>Males / females</p> <p>10/dose/sex</p> <p>Mean BW: 200 and 190 g for males and females, resp.</p> <p>At least 6-week old</p>	<p>Nitromethane</p> <p>Purity: 95.88 %</p> <p>Impurities: 3.31 % nitroethane, 0.55 % 2-nitropropane</p>	<p>Oral gavage</p> <p>Doses:</p> <p>Probe study with 500, 1000, 1500 and 2000 mg/kg bw/d</p> <p>Main test: 0, 600, 800, 1000, 1400, 1600 and 1800 mg/kg bw/d</p> <p>Vehicle: 1 % CMC</p> <p>14 d post-dosing observation period</p>	<p>LD50 (males): 1506 (IC95: 1370 – 1602) mg/kg bw</p> <p>LD50 (females): 1449 (IC95: 1261 – 1560) mg/kg bw</p> <p>LD50 males/females: 1478 mg/kg bw</p>	<p>Anonymous 16, 1980</p>

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD ₅₀	Reference
Acute oral toxicity study Not GLP-compliant No guideline Reliability 2 (according to the registration dossier)	Dogs (strain unknown) Sex not specified Varying group size	Nitromethane Purity unknown	5, 2, 6, 2 and 2 dogs were exposed to 125, 250, 500, 1000 and 1500 mg/kg bw, resp. No control group 20 % nitromethane in a 0.3 % aqueous methylcellulose solution	500<LD50<1000 mg/kg bw (concluded in the registration dossier) However, 3 out of 5 animals died at 125 mg/kg bw. DS concluded that the LD50 is < 125 mg/kg bw	Weatherby, 1955
Acute oral toxicity study Not GLP-compliant No guideline Reliability 2 (according to the registration dossier)	White male mice	Nitromethane Purity unknown	Oral gavage Doses: 5 males exposed to 1200, 1500 or 1800 mg/kg bw and 10 additional males exposed to 1500 mg/kg bw	LD50: 1440 mg/kg bw	Weatherby, 1955
Acute oral toxicity study Not GLP-compliant No guideline Reliability 2 (according to the registration dossier, however very poor quality of the PDF file)	Rabbit (strain not specified)	Nitromethane Purity unknown	Oral gavage Doses not stated: at least 750 and 1000 mg/kg bw	750<LD50<1000 mg/kg bw	Machle <i>et al.</i> , 1940
Acute oral toxicity study Not GLP-compliant No guideline Reliability 4 (according to the registration dossier)	Rat (common species) Sex not specified	Nitromethane Purity unknown	Oral gavage At least 500 and 2000 mg/kg bw	LD50: 1210 mg/kg bw	Anonymous 17, 1960

No human data or other relevant studies available.

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

A non-GLP, non-guideline, oral acute toxicity study (Anonymous 16, 1980) was conducted after a probe study. Indeed, a probe study was performed at first where SD rats (5/group) were exposed to either 0, 500,

1000, 1500 or 2000 mg/kg bw. In the exploratory study, 2 and 3 rats died within 7 days after being exposed to 1000 and 1500 mg/kg bw nitromethane, respectively. All rats died within 24 h in the group exposed to 2000 mg/kg bw, furthermore, it was noted that animals were shaky and nervous approximately 3 h after exposure. In the low dose group, no animal died.

In the main study (part one), SD rats (10/sex/dose) were exposed by oral gavage to nitromethane at doses of either 0, 600, 800, 1000, 1400 or 1800 mg/kg bw and daily observation was performed for at least 14 days. In females exposed to 1400 mg/kg bw, 4 died on day 1 and 1 died on day 3, while in males 3 died on day 1. Finally, in animals exposed to 1800 mg/kg bw, all females and 9 males died on day 1 while the last male died on day 3. No death was reported in groups of either sex exposed to 600, 800 or 1000 mg/kg bw nitromethane.

Because all animals died at the highest dose, a second experiment (part two of the main study) was performed with two doses (0 and 1600 mg/kg) to refine the LD50 with 10 animals/sex/dose. In animals exposed to 1600 mg/kg bw, 7 died on day 1 and 1 on day 4* in females and 6 in day 1 in males.

Table 9: Mortality rate

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

In the probe study, mean body weights of surviving males and females tested with any dose up to and including 1000 mg/kg bw were comparable to respective control animals. Furthermore, body weight gains in females exposed to 1400 mg/kg bw were inferior to controls (7.1 % vs. 14.6 %) but only during the first 7 days of the experiment. In the second study, performed to refine the LD50, body weight gains were inferior in males and females exposed to 1600 mg/kg during the first 7 days of experiment (24.3 % and 30.3 % in males and controls, respectively, and 6.2 and 16.4 % in females and controls, respectively) however, their body weight gains were superior from day 7 to 14 (22.8 % and 14.6 % in males and controls, respectively, and 7.8 % and 4.7 % in females and controls, respectively) in the same dose groups.

Table 10: BWG in animals exposed to 1600 mg/kg and in controls in the main study

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

Concerning clinical signs, in males exposed to 1800 mg/kg bw, hyperactivity was reported 7 hours after dosing and a bloody discharge was seen in females of the same dose group at 7 hours after dosing as well as convulsions after 13 hours. Tremors after 2 and 4 hours of dosing were objectified in animals exposed to 1600 mg/kg bw.

In the probe study, haemorrhage of intestines and/or lungs was noted during necropsy. Otherwise, gross necropsy of all animals was normal, except for 4 which had lung infections. In the second study, haemorrhage of the intestine was observed in all animals which died on day 1. All 6 survivors had normal necropsies.

The LD50 was calculated to be of 1506 (IC95: 1370 – 1602) and 1449 (IC95: 1261 – 1560) mg/kg bw in males and females, respectively. The LD50 male/female was determined to be 1478 mg/kg bw.

In an oral acute toxicity study in dogs (Weatherby, 1955), dogs were exposed by gavage to the test substance emulsified in methylcellulose (20 % solution) at doses of 125, 250, 500, 1000 or 1500 mg/kg bw (no control group). Mortality was observed daily and body weights were measured at termination. Major organs (lung, liver, spleen, kidney, stomach and small intestine) were screened microscopically and heart and adrenals were assessed only at 1500 mg/kg.

In the group exposed to 125 mg/kg bw, it was reported that one dog was euthanised at 24 h, one at 48 h and one at 72 h for unstated reasons. Their tissues were examined. The two remaining animals survived two months, then observation was terminated considering they appeared to be in good state of health. Dogs exposed to 250 mg/kg bw or more died within 30 h or were killed due to moribund state. No data on body weight is available.

Concerning the clinical signs, it was stated that in several animals, rectal temperature at termination was approximatively 40 °C.

At necropsy, swollen glomeruli, marked fatty changes and swollen proximal and distal convoluted tubules were found in kidneys in animals exposed to 1500 mg/kg bw. In the same dose group, modifications in the liver as follow: central congestion and occasional edema, cells with large nuclei and lightly stained cytoplasm with many small vacuoles, focal necrosis areas were observed. At 1000 mg/kg bw, occasional midzonal necrosis was seen in the liver 24 h after administration. In animals exposed to 500 mg/kg bw, marked fatty changes and occasional areas of haemorrhage, few chronic inflammatory cells were reported in the liver 32 h after exposure. In the three animals killed at 24 h, 48 h and 72 h after exposure to 125 mg/kg bw, slight fatty changes and few lymphocytes were seen in the liver. Regeneration was evidenced with the presence of mitotic cells in the H48 animal.

The LD50 was estimated in the registration dossier to be between 500 and 1000 mg/kg bw. However 3 out of 5 dogs died when exposed to 125 mg/kg bw, but it is not clear if the level of exposure (20 % nitromethane solution) was corrected or not.

In another oral acute toxicity study in mice (Weatherby, 1955), 5 male mice per dose were orally exposed by gavage to an aqueous solution containing 5 % nitromethane at doses of 1200, 1500 or 1800 mg/kg bw. Animals were observed for 24 h and the mortality rate was assessed. Time of death usually occurred between 8-24 hours post exposure.

Table 11: Mortality rate

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

At necropsy, small focal haemorrhage area on the ears of some animals were observed several hours after exposure.

A LD50 was set at 1440 mg/kg bw (1440 ± 110 mg/kg bw).

In an oral acute toxicity study in rabbit (Machle *et al.*, 1940), undiluted nitromethane was orally administered via gavage to rabbits. The doses were not specifically stated, but at least doses of 750 and 1000 mg/kg bw were used. After administration, the rabbits were observed for 2-3 hours before returning to their cages. When the animals survived, their weight was measured daily until they had gained back any weight loss, then the measurement was taken once or twice a week. After 20 to 40 minutes exposure, progressive weakness and collapse, unsteadiness, incoordination, ataxia and respiration modifications were reported. No significant biochemical changes in the blood were reported, nor changes in the color or in methemoglobin formation.

The LD50 was reported to be between 750 and 1000 mg/kg bw, no more information is available.

In a poorly reported oral acute toxicity test in rats (Anonymous 17, 1960), animals were orally exposed to at least 500 and 2000 mg/kg bw nitromethane and then their health status was followed up for 14 days. The LD0 and the LD100 were determined as 500 and 2000 mg/kg bw, respectively. The test substance induced narcotic effects in sublethal doses. The first effect seen was anorexia. Slight to moderate liver effects were found in animals which did not receive the lethal dose. Respiratory failure was seen in animals which died from toxic dose. All animals were necropsied for gross pathologic assessment. Lung hemorrhages and intestinal effects (gaseous fluid in intestinal tract) were found in animals which were exposed to the lethal dose. The LD50 was evaluated to be 1210 mg/kg bw.

10.1.2 Comparison with the CLP criteria

CLP criteria	Results of available studies
Acute toxicity category 4: dermal LD50: > 1000 but ≤ 2000 mg/kg bw	<p>Available studies performed with nitromethane mostly conclude on a LD50 within the classification range for acute toxicity category 4 ($300 \leq ATE \leq 2000$ mg/kg bw). In that perspective, according to table 3.1.1 of the CLP Regulation, a classification as Acute Tox. Category 4 is suggested.</p> <p>An ATE of 1450 mg/kg bw, derived from Anonymous 16 (1980), is proposed considering rats was the chosen species in the study and it is the preferable species according to the OECD test guidelines. Furthermore, the proposed ATE is the LD50 derived from the females mortality rate, considered as a little more sensitive than males.</p> <p>In the study performed on dogs (Weatherby, 1955), the LD50 was inferior to 125 mg/kg bw. Due to the numerous reporting deficiencies, the study was disregarded.</p>

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Classification as **Acute Tox. Cat. 4** is warranted for acute oral toxicity based on the available data. It is proposed to assign an **ATE of 1450 mg/kg bw** for acute oral toxicity.

10.2 Acute toxicity - dermal route

Hazard class not evaluated in this CLH dossier

10.3 Acute toxicity - inhalation route

Table 12: 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 (MMAD)	Dose levels, duration of exposure	Value LC ₅₀	Reference
--------------------------------------	--------------------------------	---	-----------------------------------	------------------------	-----------

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC ₅₀	Reference
<p>Acute inhalation toxicity study</p> <p>Similar to guideline design</p> <p>Reliability 2 (according to the registration dossier, however data poorly reported)</p>	<p>Rat (strain not specified)</p> <p>N= 10</p>	<p>Nitromethane</p> <p>Purity: unknown</p>	<p>12.75 mg/L (No control group)</p> <p>Exposure: 1 h</p> <p>Whole body exposure</p> <p>48 h observation</p>	<p>LD50: no data</p> <p>LCLo (1h): 12.75 mg/L</p>	<p>Anonymous 10, 1956</p>
<p>Acute inhalation toxicity study</p> <p>Comparable to test guideline</p> <p>Not GLP</p> <p>Reliability 2 (according to the registration dossier)</p>	<p>Wistar rats</p> <p>Group of 8-10 rats (not specified if per dose or in total)</p>	<p>Nitromethane</p> <p>Purity: unknown</p>	<p>500, 2500 and 13000 ppm (equivalent to 1.25, 6.25 and 32.5 mg/L air, resp.)</p> <p>6 h exposure</p> <p>Daily exposure 5 d/w, during 3 w at the lowest dose</p> <p>Daily exposure during 4 d at the middle dose</p> <p>And single exposure at the highest dose</p>	<p>LC100 (6h): 13000 ppm (equivalent to 32.5 mg/L)</p>	<p>Dequidt J. <i>et al.</i>, 1973</p>
<p>Inhalation toxicity study</p> <p>Not following guideline</p> <p>Not GLP-compliant</p> <p>Reliability 2 (according to the registration dossier, however poorly reported)</p>	<p>Rabbit</p> <p>N= 2/dose</p>	<p>Nitromethane</p> <p>Purity unknown</p>	<p>500 ppm (for 140 h), 1000 ppm (for 30 h), 2500 ppm (for 12 h), 5000 ppm (for 3 or 6 h), 10000 ppm (for 1, 3 or 6 h), 22500 ppm (for 1 h), 30000 ppm (for 0.25, 0.5, 1 or 2 h), 50000 ppm (for 1 h). For lower exposure, it is not stated how much time/d the animals were exposed.</p> <p>500, 1000, 2500, 5000, 10000, 22500, 30000 and 50000 ppm, approx. equivalent to 1.25, 2.50, 6.25, 12.5, 25, 56.2, 75 and 125</p>	<p>LC50 (6h): 5000 ppm (equivalent to 12.5 mg/L)</p>	<p>Machle <i>et al.</i>, 1940</p>

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC ₅₀	Reference
			mg/L, resp.		
Inhalation toxicity study Not following guideline Not GLP-compliant Reliability 2 (according to the registration dossier, however poorly reported)	Guinea pig N= 2/dose	Nitromethane Purity: unknown	500 ppm (for 140 h), 1000 ppm (for 30 or 48 h), 2500 ppm (for 12 h), 5000 ppm (for 3 or 6 h), 10000 ppm (for 1, 3 or 6 h), 22500 (for 1 h), 30000 ppm (for 0.25, 0.5, 1 or 2 h) or 50000 ppm for 1 h 500, 1000, 2500, 5000, 10000, 22500, 30000 and 50000 ppm, approx. equivalent to 1.25, 2.50, 6.25, 12.5, 25, 56.2, 75 and 125 mg/L, resp.	LC50 (6h): 5000 ppm (equivalent to 12.5 mg/L)	Machle <i>et al.</i> , 1940
Read-across applied by the registrant Acute inhalation toxicity study Not following guidelines Not GLP-compliant Reliability 2 (according to the registrant)	Wistar rats Females N= 6/dose Mean BW: 150-200g	1-nitropropane (EC: 203-544-9; CAS: 108-03-2) Purity: 96.12 %	8.60, 11.02 and 14.40 mg/L 1h exposure 7d post exposure observation period	LC50 (1h): 11.02 mg/L (equivalent to 5.51 mg/L for 4 h of exposure) Mortality: 0, 3 and 5 females respectively at 8.60, 11.02 and 14.40 mg/L (1 h after exposure)	Anonymous 10, 1956

No human data or other relevant studies available.

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

In an acute inhalation toxicity study in rat (Anonymous 10, 1956), 10 rats were exposed through inhalation to 12.75 mg/L air nitromethane for one hour. An observation period of 48 h was followed. No death was reported. The LC₅₀ was then higher than 12.75 mg/L. Concerning the clinical signs, mild sedation and eye irritation were reported in animals during exposure and recovery periods.

In another acute inhalation toxicity study in rat (Dequidt *et al.*, 1973), animals were exposed to vapours of nitromethane during 6 hours per day. The dose and duration varied as follow:

Table 13: duration and dose

Experiment	Dose (ppm)	Approx.	Duration
1	13000	32.5 mg/L	Once
2	2500	6.25 mg/L	4 days
3	500	1.25 mg/L	3 weeks – 5 days per week

The number of animals that were used is not very clear. Groups of 8-10 rats is mentioned but it is not stated if 8-10 rats were selected for each dose or in total.

In the first experiment, all rats died after 6 h when exposed once to 13000 ppm. In the second experiment, the rats died after the 4th day of exposure to 2500 ppm. All animals exposed to the lowest dose survived until euthanasia, after 3 weeks of exposure. The LC100(6h) was set at 13000 ppm. The LC50 could not be determined.

In the second experiment, no methemoglobin was detected in the blood. Furthermore, nitromethane was not found in the tissue examination. However, the nitrite ion concentration was concluded to be at a high level. Animals exposed to 500 ppm appeared comparable to control after 3 weeks of exposure.

The methemoglobin was measured as well as the concentration of nitromethane in the liver, lungs, heart and kidney. Based on the results, nitromethane does not seem to induce methemoglobinemia (<1 %). Nitromethane was only found in the liver of animals exposed to 13000 ppm (0.27 g/100 g).

Table 14: Effects seen in rats after exposure to nitromethane

Dose (ppm)	Nb of exposures	MetHb (%)	NO2 (µg /100 g organ)				
			Heart	lungs	kidney	Liver	Spleen
500	15	0-0.25	Trace	Trace	Trace	Trace	Trace
2500	4	0	625	372	408	Trace	800
13000	1	0-0.1	554	686	311	Trace	350

In an acute inhalation toxicity study in rabbit (Machle *et al.*, 1940), rabbits were exposed to doses of nitromethane, during various amounts of time according to the dose: 500 ppm (140 h), 1000 ppm (30 h), 2500 ppm (12 h), 5000 ppm (3 or 6 h), 10000 ppm (1, 3 or 6 h), 22500 ppm (1 h), 30000 ppm (0.25, 0.5, 1 or 2 h), 50000 ppm (1 h). The doses of 500, 1000, 2500, 5000, 10000, 22500, 30000 and 50000 ppm, were approximatively equivalent to 1.25, 2.50, 6.25, 12.5, 25, 56.2, 75 and 125 mg/L, respectively. For lower exposure levels, it is not stated how much time/d the animals were exposed. The maximal concentration used was 5 %. An observation period of at least 2 months was followed. The animals were then necropsied. Body weights were measured before the experiment and then daily. Blood tests were performed daily prior to the experiment and then weekly, red and white blood cells as well as hemoglobin percentage were counted. Surviving animals were followed for 2 months after exposure. Control rabbits were followed for a 6-month period.

All animals died in the group exposed to 10000 ppm for 6 h, 30000 ppm for 2 h or 50000 ppm for 1 h. Half of the animals per group died after being exposed to 5000 ppm for 6 h. No animal died after being exposed to 10000 ppm for 3 h or 30000 ppm for 1 h.

Table 15: Number of deaths at each dose level:

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

Concerning the clinical signs, irritation, convulsion, jerking or twitching in extremities, slight and transient loss of body weight were reported. Stupor, narcosis and slight general anesthesia were observed when animals were exposed to more than 3 % nitromethane in air.

Necropsy findings showed general visceral and cerebral congestion in all animals (but less marked in controls). When exposed to high concentrations, upper respiratory irritation and acute pulmonary congestion were observed. Liver damage was evidenced in all animals. Furthermore, liver damage of different severity was seen in all animals. Edema, cloudy swelling or pallor were reported in kidneys or myocardium, among others, and it was considered as changes seen in animals after exposed to lethal dose.

- LC100 (1h): 50000 ppm; LC100 (2h): 30000 ppm; LC100 (6h): 10000 ppm
- LC50 (6h): 5000 ppm (± 12.5 mg/L)
- LC0 (1h): 30000 ppm; LC0 (3h): 10000 ppm;

In an acute inhalation toxicity study in guinea pig (Machle *et al.*, 1940), animals were exposed to nitromethane as different doses (500, 1000, 2500, 5000, 10000, 22500, 30000 and 50000 ppm, approximatively equivalent to 1.25, 2.50, 6.25, 12.5, 25, 56.2, 75 and 125 mg/L, respectively), for different times of exposure (see Table 16), and then a 2-month observation period was followed. LC50(6h) was determined to be 5000 ppm (12 mg/L).

Table 16: Duration and doses of exposure

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

All animals exposed to 50000 and 30000 ppm died after 1 h of exposure. All animals died after being exposed to 10000 ppm for 6 h, but none died after 1h exposure. 1 out of 2 guinea pigs died after 6h expose of 5000 ppm nitromethane. Finally, no animals died after being exposed to 500 ppm nitromethane for 140 h.

Table 17: Number of dead animals per dose level

Dose (ppm)	500	5000	10000	10000	30000	50000
Exposure (h)	140	6	1	6	1	1
Mortality	0/2	1/2	0/2	2/2	2/2	2/2

Irritation, convulsions, jerking/twitching in extremities, light transient loss of weight were reported. When animals were exposed to more than 3 % nitromethane in air, stupor, narcosis, slight general anesthesia were seen.

General visceral and cerebral congestion was reported in all animals (but less marked in controls). When exposed to high concentrations, upper respiratory irritation and acute pulmonary congestion were observed. Liver damage was evidenced in all animals. Furthermore, liver damage of different severity was seen in all animals. Edema, cloudy swelling or pallor were reported in kidneys or myocardium, among others, and it was considered as changes seen in animals after exposed to lethal dose.

- LC100(1h): 50000 ppm; LC100(1h): 30000 ppm; LC100(6h): 10000 ppm
- LC50(6h): 5000 ppm (≈12.5 mg/L)
- LC0(1h): 10000 ppm; LC0(140h): 500 ppm

In a supporting study based on 1-nitropropane (Anonymous 10, 1956), groups of 6 female rats were exposed during 1 h to vapours of 1-nitropropane (8.60, 11.02 or 14.40 mg/L). An observation period of 7 d was followed. Irritation was reported after 5 min of exposure. Ataxia was observed in all animals after removal of the exposure chamber and normal movement came back after 24 h. 0, 3 and 5 females died 1 h after exposure respectively at 8.60, 11.02 and 14.40 mg/L. The LC50 was set at 11.02 mg/L air 1-nitropropane after 1 h of exposure. This corresponds to a LC50 of 7.55 mg/L air nitromethane after 1 h of exposure.

10.3.2 Comparison with the CLP criteria

In Anonymous 10 (1956), rats were exposed to only one dose (12.75 mg/L air) for one hour. No animals died after this single exposure. The corrected level for a 4-h exposure is approximately 3.20 mg/L nitromethane. This level was determined by the registrant as the lowest concentration resulting in fatality (LCLo) but it cannot be used as such to derive a classification usually based on the LD50.

In Dequidt *et al.* (1973), a lethal concentration inducing a 100 % death rate after 6-h of exposure was determined at 32.5 mg/L in Wistar rats. The corrected level for a 4-h exposure is approximately of 48.75 mg/L. The available data is not sufficient to correctly conclude on this study.

Machle *et al.* (1940) set a lethal concentration inducing a 50 % death rate (LC50) at 12.5 mg/L in rabbits and Guinea pigs after a 6-h exposure. The corrected level for a 4-h exposure results in a LC50 of 18.75 mg/L, for both species. The DS notes that the available data was of very low quality and the reliability of this study was therefore downgraded.

A supporting study from Anonymous 10 (1956) with 1-nitropropane concluded of a LC50 at 11.02 mg/L air for a 1-h exposure. Three different doses were tested, unlike the nitromethane part of the study from the same authors. The calculated level of exposure would be 5.51 mg/L air for a 4-h exposure. The provided data for this study was more detailed than for nitromethane (Anonymous 10, 1956), e.g. purity of the test substance, strain of rats, BW at start, etc..

According to the CLP criteria, a substance (vapour) should be classified as **Acute Tox. Category 3** for the inhalation route when the ATE is estimated to be between $2.0 \leq \text{ATE} \leq 10.0$ mg/L. The mentioned LC50 by Anonymous 10 (1956) in rats (11.02 mg/L air for a 1h-exposure; 5.51 mg/L for a 4-h exposure) is in line with those criteria and is selected by the DS. The ATE proposed is **5.50 mg/L**. In Machle *et al.*, the number of animals was insufficient to correctly assess this endpoint. The lower amount of data available in Anonymous 10 (1956) and in Dequidt (1973) reduced its reliability as explained above.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

A classification as **Acute Tox. 3, H331** is proposed. Based on CLP Regulation, an ATE of **5.50 mg/L (vapour)** is warranted.

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 18: 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
NITROMETHANE				
<i>In vitro</i> gene mutation test in bacteria OECD TG 471 Deviation: 4 instead of 5 strains Non-GLP Reliability 2 (according to the registration dossier)	Nitromethane Purity: > 99 %	Pre-incubation test Strain: 4 <i>S. typh.</i> strains (TA98, TA100, TA1535 and TA1537) Test conc.: 100, 333.3, 1000, 3333.3 and 10000 µg/plate. +- S9 Vehicle: DMSO	No sign increase in the frequency of revertant colonies up to 10 mg/plate, +- S9 Only in TA100, cytotoxicity was observed at the highest conc tested. Negative	Mortelmans <i>et al.</i> , 1986
<i>In vitro</i> 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 <i>S. typh.</i> strains (TA98, TA100, TA1535, TA1537 and TA1538) Test conc.: A conc resulting in saturated vapour atmosphere (47465 ppm) caused cytotoxicity in strains TA1535 and TA1537. For this reason, a conc of 23732 ppm (118.7 mg/L) was tested. +- S9	No sign increase in the frequency of revertant colonies at 23732 ppm, +- S9 Negative	Anonymous 27, 1980
<i>In vitro</i> 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 conc ➤ 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	Negative +- S9 at conc as high as the limit conc of 4980 µg/mL Negative	NTP, 1997

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		+ - S9 Vehicle: distilled water		
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 conc ➤ 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 conc as high as the limit conc 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 abstract available to the DS)	Nitromethane Purity unknown	Strains: 3 <i>S. typh.</i> strains (TA1535, TA1537 and TA1538) and 1 <i>Saccharomyces cerevisiae</i> (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 deficiencies)	Nitromethane Purity unknown	Strain: 3 <i>S. typh.</i> strains (TA98, TA100 and TA102) Test conc: Not specified but up to 200 µmol/plate. Only without S9 Vehicle: not specified	Negative without S9	Dayal <i>et al.</i> , 1989

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
<p>In vitro cell transformation study in mammalian cells</p> <p>EU Method B.21</p> <p>Non-GLP</p> <p>Reliability 2 (according to the registration dossier)</p>	<p>Nitromethane</p> <p>Purity unknown</p>	<p>cells type: Syrian hamster embryo (SHE)</p> <p>Test conc.: 2000, 2500, 3000, 3500, 4000 and 5000 µg/mL (= top dose)</p> <ul style="list-style-type: none"> ➤ Exposure for 24 h followed by 6-7 d of growth ➤ Exposure for 7 d <p>Vehicle: DMSO</p>	<p>A dose-dependent sign increase in the morphological transformation frequency seen at the two highest conc tested.</p> <p>Positive</p>	<p>Kerckaert <i>et al.</i>, 1996</p>
<p>In vitro micronucleus test in SHE cells</p> <p>Non-GLP</p> <p>Reliability 2 (according to the registration dossier)</p>	<p>Nitromethane</p> <p>Purity unknown</p>	<p>Cells type: SHE cells</p> <p>Met. act.: not used</p> <p>Test conc:</p> <ul style="list-style-type: none"> - With DMSO: 0, 5.0, 5.5 and 6.0 µg/mL - With Media: 0, 3500, 4000, 5000 (µg/mL) <p>Vehicle: DMSO or media</p>	<p>Nitromethane did not induce an increased frequency of micronuclei in SHE cells.</p> <p>Negative</p>	<p>Gibson <i>et al.</i>, 1997</p>
		<p>Results of an additional <i>in vitro</i> 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.</p>		
NITROETHANE				
<p>In vitro gene mutation test in bacteria</p> <p>OECD TG 471</p> <p>Non-GLP</p> <p>Reliability 2 (according to the registration dossier)</p>	<p>Nitroethane</p>	<p><i>S. typh.</i></p> <p>Deviations: only 4 out of 5 strains used (TA98, TA100, TA1535, TA1537)</p> <p>Test conc: 100, 333.3, 1000, 3333.3 and 10000 µg/plate ± S9</p> <p>Vehicle: DMSO</p> <p>No neg control</p> <p>Pos controls:</p>	<p>Cytotoxicity observed in all 4 strains at 10000 µg/plate</p> <p>Precipitation was observed in the highest conc tested in most experiments in all the strains</p> <p>Pos control: induced a clear increase in the number of revertants</p> <p>No sign increase in the frequency of revertant colonies was observed for any of the bacterial strains with</p>	<p>Mortelmans <i>et al.</i>, 1986</p>

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		-S9: 4-nitro-o-phenylenediamine for TA98, sodium azide for TA100 and TA1535 and 9-aminoacridine for TA1537 +S9: 2-aminoanthracene	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	<i>S. typh</i> 5 strains (TA98, TA100, TA1535, TA1537 and TA1538) Conc.: 55450 ppm (vapours) and at least 27725 ppm No vehicle Neg control: unspecified Pos 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 conc of 27725 ppm was tested. No sign increase in the frequency of revertant colonies Negative	Anonymous 29, 1980
In vitro gene mutation study in mammalian cells OECD TG 476 GLP-compliant Reliability 1 (according to the registration dossier)	Nitroethane	Cells type: CHO Target gene: HGPRT 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	No cytotoxicity observed at the highest conc tested Nitroethane was non-mutagenic both in absence and in presence of S9 metabolic fraction in the mammalian gene mutation test at conc up to the limit conc. Negative	Anonymous 30, 2012
In vitro gene mutation study in bacteria OECD TG 471 Non-GLP Reliability 2 (according to the	Nitroethane	<i>S. typh.</i> 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 conc.	Neg without met. act. No cytotoxicity observed at any conc. Negative	Dayal <i>et al.</i> , 1989

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
registration dossier, however reporting deficiencies)		Vehicle: DMSO + phosphate buffer (0.2 M, pH 7.4) Without met. act.		
1-NITROPROPANE				
<i>In vitro</i> 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	<i>S. typh.</i> TA98, TA100, TA1535 and TA1537 and <i>E. Coli</i> WP2uvrA- Test conc.: 20, 150, 500, 1500 and 5000 µg/plate	Cytotoxicity: no Genotoxicity: negative	Anonymous 31, 1996
<i>In vitro</i> 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
<i>In vitro</i> 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 <i>et al.</i> , 1988
<i>In vitro</i> gene mutation test in	1-nitropropane	Chinese hamster lung cells (V79)	Cytotoxicity: yes	Roscher <i>et</i>

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
<p>mammalian cells</p> <p>OECD TG 476</p> <p>Not GLP</p> <p>Reliability 2 (according to the registration dossier, however only summary available, not enough information to confirm the validity of the study)</p>	<p>Purity: 97.4 %</p> <p>Impurity: 2-nitropropane (2.3 %)</p>	<p>Test conc.: 0, 0.3, 1, 3, 6 and 10 mM</p>	<p>Genotoxicity: positive</p>	<p><i>al.</i>, 1990</p>
<p>In vitro micronucleus test in mammalian cells</p> <p>OECD TG 487</p> <p>Not GLP</p> <p>Reliability 2 (according to the registration dossier, however only summary available, not enough information to confirm the validity of the study)</p>	<p>1-nitropropane</p> <p>Purity: 97.4 %</p> <p>Impurity: 2-nitropropane (2.3 %)</p>	<p>Chinese hamster lung cells (V79)</p> <p>Test conc.: 0, 0.3, 1, 3, 6 and 10 mM</p>	<p>Cytotoxicity: yes</p> <p>Genotoxicity: positive</p>	<p>Roscher <i>et al.</i>, 1990</p>
<p>In vitro DNA damage and/or repair study</p> <p>OECD TG 476</p> <p>Not GLP</p> <p>Reliability 2 (according to the registration dossier, however only summary available, not enough information to confirm the validity of the study)</p>	<p>1-nitropropane</p> <p>Purity: 97.4 %</p> <p>Impurity: 2-nitropropane (2.3 %)</p>	<p>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)</p> <p>Test concentrations: 0.1 – 10 mM</p>	<p>Cytotoxicity: unspecified</p> <p>Genotoxicity: negative</p>	<p>Andrae U. <i>et al.</i>, 1988</p>
<p>In vitro gene mutation test in bacteria</p> <p>With and without met. act.</p> <p>OECD TG 471</p>	<p>1-nitropropane</p> <p>Purity: ~99 %</p> <p>Vehicle: DMSO</p>	<p><i>S. typh</i> TA98, TA100, TA1535 and TA1537 and <i>E. Coli</i> WP2uvrA-</p> <p>Test conc.: 0, 8, 40, 200, 1000 and 5000 µg/plate (first experiment) and 0, 312.5, 625, 1250, 2500 and 5000 µg/plate</p>	<p>Cytotoxicity: no</p> <p>Genotoxicity: negative</p>	<p>Anonymous 33, 1994</p>

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
GLP Reliability 1 (according to the registration dossier)				
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 confirm the validity of the study)	1-nitropropane Purity: 97 % Vehicle: DMSO	<i>S. typh.</i> TA98, TA100, TA1535 and TA1537 Conc.: 0, 100, 333, 1000, 3333 and 10000 µg/plate	Cytotoxicity: no Genotoxicity: negative	Haworth S. <i>et al.</i> , 1983

Table 19: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
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 conc up to 1500 ppm. Negative	NTP, 1997
		Due to very poor quality of the copy, the study		Gocke <i>et al.</i> , 1981

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		will not be presented in the CLH report and will not be assessed.		
NITROETHANE				
<p>In vivo micronucleus test</p> <p>Prior to OECD TG 474</p> <p>Prior to GLP</p> <p>CD-1 mice (Charles River)</p> <p>14/sex/ in control groups</p> <p>8/sex/dose</p> <p>Reliability 2 (according to the registration dossier, however study not available to the DS)</p>	<p>Nitroethane</p>	<p>Oral (gavage)</p> <p>2x/day</p> <p>Doses: 0.25, 0.5 or 1.00 mL/kg bw/d (highest dose = half the oral LD50 value)</p> <p>Sacrifice 6 h after the last dose</p> <p>Vehicle: unknown</p> <p>Concurrent control: tap water</p> <p>Positive control: methylmethanesulfonate (90 mg/kg bw/d, IP route)</p>	<p>No sign increase in the frequency of micronucleated polychromatic erythrocytes, at doses up to 1 mL/kg bw/d, in either sex.</p> <p>Negative</p>	<p>Hite <i>et al.</i>, 1979</p>
1-NITROPROPANE				
<p>In vivo micronucleus test</p> <p>No guideline followed</p> <p>Not GLP</p> <p>Reliability 2 (according to the registration dossier, however DS not access to raw data)</p>	<p>1-nitropropane</p> <p>Purity: unspecified</p>	<p>Male SD rats (4-8/groups)</p> <p>Gavage</p> <p>Single dose</p> <p>Bone marrow: 24 h: 100, 200, 300 and 400 mg/kg; 48h: 100, 200 and 300 mg/kg</p> <p>Liver: 72 h: 300 mg/kg (lethality observed at 500 mg/kg)</p>	<p>Genotoxicity: negative in the bone marrow, however positive in liver</p> <p>Toxicity: yes lethality at 500 mg/kg bw</p>	<p>George <i>et al.</i>, 1989</p>
<p>In vivo mammalian cell study : DNA damage and/or repair</p> <p>No guideline followed</p> <p>Not GLP</p> <p>Reliability 2 (according</p>	<p>1-nitropropane</p> <p>Purity: 97.4 %</p> <p>Vehicle: olive oil</p>	<p>Wistar rats</p> <p>9 controls and 2/sex after 1 h and 17 h</p> <p>IP, single injection</p> <p>Conc.: 20, 40, 60 and 80 mg/kg</p>	<p>Genotoxicity: negative</p>	<p>Andrae <i>et al.</i>, 1988</p>

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
to the registration dossier, however DS not access to raw data)				
<p><i>In vivo</i> mammalian somatic cell study: cytogenicity/erythrocyte micronucleus</p> <p>No guideline followed</p> <p>GLP compliance unspecified</p> <p>Reliability 2 (according to the registration dossier, however poor quality of the PDF file, difficult to analyse the data)</p>	<p>1-nitropropane</p> <p>Purity: unspecified</p>	<p>Mouse (5/sex/group)</p> <p>IP, single dose</p> <p>Conc.: no information available</p>	<p>Genotoxicity: negative</p>	<p>Kliesch and Adler, 1987</p>

No human data available

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

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 20: 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 \pm 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 $\mu\text{g/mL}$. 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 21: Chromosomal aberration in CHO cells

Compound	Dose level ($\mu\text{g/mL}$)	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 $\mu\text{g/mL}$. 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 22: SCE assay results in CHO cells

Dose level ($\mu\text{g/mL}$)	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 (Kerckear *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 23: 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

CLH REPORT FOR NITROMETHANE

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 than 33 % of the nucleus were taken into account. The test results were negative, with either vehicle.

Table 24: 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 25: 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

CLH REPORT FOR NITROMETHANE

	+ 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 26: CHO cells survival (N colonies/plate) after exposure to nitroethane 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

CLH REPORT FOR NITROMETHANE

	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 colonies/plate) in the treated group/(mean number of colonies/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 27: 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 colonies/plate	mutant	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 28: Mutation assay results (with S9), in the initial test

Dose (µg/mL)	Assay	Mutation result		Cloning efficiency (CE)				Mutants per million clonable cells
		Total colonies/plate	mutant	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

CLH REPORT FOR NITROMETHANE

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

Dose (µg/mL)	Assay	Mutation result Total mutant colonies/plate	Cloning efficiency (CE)				Mutants per million clonable cells
			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 30: Mutation assay results (with S9) in the confirmatory test

Dose (µg/mL)	Assay	Mutation result Total mutant colonies/plate	Cloning efficiency (CE)				Mutants per million clonable cells
			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 31: 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 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 32).

Table 32: 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: 6 h 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 33)

Table 33: 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	NE	312.5	4/200	625	4/200	625	NE
625	5/200	625	8/200	1250	10*/200	1250	2/200
1250	7/200	1250	8/200	2500	5/200	2500	0/200
2500	7/200	2500	toxic	5000	Toxic	5000	3/200
MMC	65***/150	MMC	97***/100	CP	4/200	CP	78***/100

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

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 approximately of 11, 18, 31, 53 and 46 $\times 10^6$ 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 approximately 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 $\times 10^3$, 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 $\mu\text{g}/\text{plate}$ for the first experiment and 0, 312.5, 625, 1250, 2500 and 5000 $\mu\text{g}/\text{plate}$ 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 34 and Table 35)

Table 34: Number of revertants (number of colonies/plate) (experiment 1)

Conc. (in µg/plate)	Without met. act.					With met. act.				
	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 35: Number of revertants (number of colonies/plate) (experiment 2)

Conc. (in µg/plate)	Without met. act.					With met. act.				
	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 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 36: 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.

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 37).

Table 37: Incidence of micronuclei and PCE

Experiment	A		B
Sampling time	24 h	48 h	24 h

CLH REPORT FOR NITROMETHANE

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.

10.8.2 Comparison with the CLP criteria

CLP criteria cat. 1	CLP criteria cat. 2
<p>Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans.</p> <p>Substances known to induce heritable mutations in the germ cells of humans.</p> <p>The classification in Category 1A is based on positive evidence from human epidemiological studies.</p> <p>Substances to be regarded as if they induce heritable mutations in the germ cells of humans.</p> <p>The classification in Category 1B is based on:</p> <ul style="list-style-type: none"> – positive result(s) from <i>in vivo</i> heritable germ cell mutagenicity tests in mammals; or – positive result(s) from <i>in vivo</i> 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 <i>in vivo</i>, 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. 	<p>Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans.</p> <p>The classification in Category 2 is based on:</p> <ul style="list-style-type: none"> – Positive evidence obtained from experiments in mammals and/or in some cases from <i>in vitro</i> experiments, obtained from: <ul style="list-style-type: none"> – Somatic cell mutagenicity tests <i>in vivo</i>, in mammals; or – Other <i>in vivo</i> somatic cell genotoxicity tests which are supported by positive results from <i>in vitro</i> mutagenicity assays. <p>Note: Substances which are positive in <i>in vitro</i> 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.</p>

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* unscheduled 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/hgp^{rt}) 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 evidence 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 increase 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 38: Summary data regarding *in vitro* tests

<i>In vitro</i>				
Test Guidelines	Substances	Results	References	Remarks
OECD TG 471	NM	Negative	Mortelmans <i>et al.</i> , 1986	/
	NM	Negative without S9	Dayal <i>et al.</i> , 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 <i>et al.</i> , 1986	/
	NE	Negative without	Dayal <i>et al.</i> , 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 <i>et al.</i> , 1983	/
OECD TG 473	NM	Negative	NTP, 1997	/
OECD TG 476	NE	Negative	Anonymous 30, 2012	/
	1-NP	Positive	Roscher <i>et al.</i> , 1990	Cytotoxicity : yes
	1-NP	Negative	Andrae <i>et al.</i> , 1988	/
OECD TG 479	NM	Negative	NTP, 1997	/
OECD TG 482	1-NP	Negative	Andrae <i>et al.</i> , 1988	/
OECD TG 487	1-NP	Positive	Roscher <i>et al.</i> , 1990	Cytotoxicity: yes
EU method B.21	NM	Positive	Kerckaert <i>et al.</i> , 1996	/
No guideline - micronucleus test in SHE cells	NM	Negative	Gibson <i>et al.</i> , 1997	/
No guideline - chromosome aberration study in mammalian cells	1-NP	Negative	Anonymous 33, 1994	/

Table 39: Summary data regarding *in vivo* tests

<i>In vivo</i>				
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 <i>et al.</i> , 1989	/
No guideline mammalian cell study : DNA damage and/or repair	1-NP	Negative	Andrae <i>et al.</i> , 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 40: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
NITROMETHANE			
<p>Long term inhalation study</p> <p>Similar to OECD TG 451</p> <p>GLP-compliant</p> <p>2 years</p> <p>Rats,</p> <p>F344/N</p> <p>50/sex/dose</p> <p>Reliability 1 (according to the registration dossier)</p>	<p>Nitromethane</p> <p>Purity: > 99 %</p> <p>inhalation</p> <p>6 h/d, 5 d/w</p> <p>0, 94, 188, 375 ppm (approx. equivalent to 0, 0.235, 0.47 and 0.94 mg/L, resp.)</p>	<p>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.)</p> <p>Clinical signs: masses on shoulders and torso consistent with mammary gland neoplasms</p> <p>BWG: slightly increased in females exposed to 375 ppm vs. controls</p> <p>Organ weight: no data</p> <p>Histopathology:</p> <ul style="list-style-type: none"> - 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 <p>Neoplastic effects:</p> <p>In females: Mammary gland, out of 50 animals and at 0, 94, 188 and 375, resp. (%):</p> <ul style="list-style-type: none"> - 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 %) 	NTP, 1997
<p>Long term inhalation study</p> <p>Equivalent or similar to OECD TG 451</p>	<p>Nitromethane</p> <p>Purity: > 99 %</p> <p>Impurities: 0.25 % nitroethane, 0.03 % 2-</p>	<p>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</p> <p>Clinical sign: in the eyes, swelling and exophthalmos coincident with harderian gland tumours, in both sexes</p>	NTP, 1997

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
<p>GLP-compliant 2 years Mice, B6C3F1 50/sex/group Reliability 1 (according to the registration dossier)</p>	<p>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.)</p>	<p>BWG: no effects in males, slightly increased BW in females during the study but similar to controls at study termination</p> <p>Organ weights: no data</p> <p>Histopathology:</p> <ul style="list-style-type: none"> - Sign. increased incidence olf. epith. degeneration in both sexes, in all treated groups - Sign. increase in olf. epith. metaplasia in both sexes at 375 and 750 ppm - Sign. increase in resp. epith. hyaline degeneration in all treated groups in females and at the middle and high doses in males. <p>Neoplastic effects</p> <p>- Harderian gland: Male and female:</p> <p>Adenoma (%):</p> <p style="padding-left: 20px;">M: 9/50 (18), 10/50 (20), 19/50 (38)**, 32/50 (64)** (HCD: 2-14 %)</p> <p style="padding-left: 20px;">F: 5/50 (10), 7/50 (14), 16/50 (32)**, 19/50 (38)** (HCD: 0-16 %)</p> <p>Carcinoma (%):</p> <p style="padding-left: 20px;">M: 1/50(2), 1/50 (2), 6/50 (12), 5/50 (10) (HCD: 0-4 %)</p> <p style="padding-left: 20px;">F: 1/50 (2), 2/50 (4), 4/50 (8), 3/50 (6) (HCD: 0-4 %)</p> <p>Adenoma or carcinoma (%):</p> <p style="padding-left: 20px;">M: 10/5 (20), 11/50 (22), 25/50 (50)**, 37/50 (74)** (HCD: 2-14 %)</p> <p style="padding-left: 20px;">F: 6/50 (12), 9/50 (18), 20/50 (40)**, 21/50 (42)** (HCD: 0-16 %)</p> <p>- Liver: Female (%):</p> <p>Hepatocellular adenoma:</p> <p style="padding-left: 20px;">F: 14/50 (28), 25/49 (51)*, 17/49 (35), 35/50 (70)** (HCD: 0-40 %)</p> <p>Hepatocellular carcinoma:</p> <p style="padding-left: 20px;">F: 10/50 (20), 14/49 (29), 8/49 (16), 12/50 (24) (HCD: 2-30 %)</p>	

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		<p>Hepatocellular adenoma or carcinoma: F: 19/50 (48), 34/49 (69)**, 22/49 (45), 40/50 (80)** (HCD: 6-54 %)</p> <p>No increase in liver tumours was observed in Males.</p> <p>Lung: Male and female (%):</p> <p>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 %)</p> <p>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 %)</p> <p>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 %)</p>	
<p>Long term inhalation toxicity study</p> <p>Rats / Long-Evans / male + female</p> <p>40 animals/group</p> <p>OECD TG 451</p> <p>GLP not specified</p> <p>Reliability 1 (according to the registration dossier)</p> <p>Major deviations from OECD TG 451 guideline:</p>	<p>Nitromethane</p> <p>Purity: 96.26 %</p> <p>Impurities: 2.79 % nitroethane, 0.62 % 2-nitropropane</p> <p>Inhalation</p> <p>Doses: 0, 100, 200 ppm (approx. equivalent to 0, 0.25 and 0.50 mg/L, resp.)</p> <p>Duration of exposure: 7 h/d, 5 d/w for 103 w</p>	<p>Mortality: 37.5, 42.5 and 37.5 % of males and 25, 27.5 and 40 % of females died</p> <p>Body weights: - similar to controls in males, - sign. lower than controls in females after 1 year exposure at 100 and 200 ppm</p> <p>Clinical chemistry: no clinically significant effects in either sex</p> <p>Hematology: no effects in either sex</p> <p>Organ weights (brain, liver, kidneys, lungs, heart): no effects in relative and absolute weights, in both sexes</p> <p>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.</p> <p>Neoplastic effects:</p>	<p>Anonymous 34, 1990</p>

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
<ul style="list-style-type: none"> - only 2 doses were tested - 40 animals / group - some tissues were not examined microscopically (parathyroid, epididymis, caecum, rectum, bone marrow,...) 		<ul style="list-style-type: none"> - 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			
<p>Long term inhalation toxicity study</p> <p>2 years</p> <p>Similar to OECD TG 453</p> <p>GLP compliant: not specified</p> <p>Rat</p> <p>Long-Evans</p> <p>40/group (control & 100 ppm)</p> <p>41 males & 39 females (200 ppm)</p> <p>Reliability 2 (according to the registration dossier)</p> <p>Major deviations:</p> <ul style="list-style-type: none"> - only 2 doses tested - 40 animals/group - some tissues were not 	<p>Nitroethane</p> <p>Purity: 97.92 %</p> <p>Impurities: nitromethane 0.01 % and 2-nitropropane 2.07 %</p> <p>Inhalation</p> <p>7 h/d, 5 d/w</p> <p>Conc.: 0, 100, 200 ppm (corresp. approx. to 0, 0.31 and 0.61 mg/L, resp.)</p>	<p>Mortality: no treatment-related effect</p> <p>BW: sign. ↓ at 100 ppm in males and at 200 ppm in females</p> <p>Clinical chemistry: slight but sign. ↑ of tot. prot. and BUN in females exposed to 200 ppm</p> <p>Hematology: No effects observed. Methb levels not assessed.</p> <p>Organ weights (brain, liver, kidneys, lungs, heart): no treatment-related effect</p> <p>Histopathology: no effect</p> <p>Neoplastic effects:</p> <ul style="list-style-type: none"> - 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 	<p>Anonymous 35, 1986</p>

CLH REPORT FOR NITROMETHANE

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

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Not-GLP Reliability 2 (according to the registration dossier, however only summary available to the DS)			
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 <i>et al.</i> , 1968

No human data or other relevant information available.

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

Data on Nitromethane

In a long term inhalation toxicity 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 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 Total (% ± St. Dev.) Range
Males	No tumours reported					
	Adenoma (%)	2/50 (4)	0/50	0/50	2/50 (4)	3/348 (0.9 ± 1.6 %) 0-4 %
Females	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 appear 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 inhalation toxicity 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 h/d, 5 d/week). The doses of 0, 188, 375 and 750 ppm were approximately 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. Coincidentally 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 nitromethane

Exposure level (ppm)	0	188	375	750
Male (%)	19/50 (38)	14/50 (28)	20/50 (40)	21/50 (42)

Female (%)	25/50 (50)	22/50 (44)	24/50 (48)	14/50 (28)
------------	------------	------------	------------	------------

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	Males	0/50	10/49**	50/50**	50/50**
	Females	0/50	22/49**	50/50**	50/50**
O.E. metaplasia	Males	0/50	1/49	41/50**	49/50**
	Females	0/50	2/49	46/50**	48/50**
R.E. hyaline degeneration	Males	5/50	5/49	50/50**	50/50**
	Females	16/50	39/49**	50/50**	50/50**

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

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 nitromethane

Dose level exposure (ppm)			0	188	375	750	HCD ^a Total (% ± 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 %)

CLH REPORT FOR NITROMETHANE

							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 carcinoma	M (%)	13/50 (26)	13/50 (26)	12/50 (24)	20/50 (40)	108/448 (24.1 ± 9.5 %) 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			
	Adenocarcinoma	0	0	1
	Myosarcoma	1	0	1
Thyroid	Adenoma C-cell	1	0	2
Pituitary gland	Adenoma	26	26	24
Liver	Meta. Primary mesenchymal	0	2	1

Malign tumours in bold

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)

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 (approximately 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 58 and Table 59). Necropsy did not reveal any treatment-related organ weight changes, and only infrequent findings were observed amongst control and exposed groups.

Table 58: 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

() : number of animals examined, ^a: compared to 21.5 m controls

Table 59: 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

() : number 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 60 and Table 61). 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 Tables 62 and 63).

Table 60: Incidence (inc.) of pituitary adenoma

	Tot. inc.	1 m	3 m	12 m	18 m

CLH REPORT FOR NITROMETHANE

		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 61: 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 62: 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 63: 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.

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

CLH REPORT FOR NITROMETHANE

		<p>Increased incidence of neoplastic effects in the lung of both sexes</p> <p>Alveolar/bronchiolar adenoma:</p> <p>M: 11/50 (22), 10/50 (20), 9/50 (18), 12/50 (24) (HCD: 6-36 %)</p> <p>F: 3/50 (6), 3/50 (6), 2/49 (4), 9/50 (18) (HCD: 0-14 %)</p> <p>Alveolar/bronchiolar carcinoma:</p> <p>M: 2/50 (4), 3/50 (6), 3/50 (6), 11/50 (22)** (HCD: 0-16 %)</p> <p>F: 0/50 (0), 3/50 (6), 5/49 (10), 3/50 (6) (HCD: 0-6 %)</p> <p>Alveolar/bronchiolar adenoma or carcinoma:</p> <p>M: 13/50 (26), 13/50 (26), 12/50 (24), 20/50 (40) (HCD: 10-42 %)</p> <p>F: 3/50 (6), 6/50 (12), 6/49 (12), 12/50 (24)* (HCD: 0-16 %)</p>		
OECD TG 451	NM	<p>Non treatment-related effects in all animals: bronchitis, glomerulosclerosis, calcification of the kidneys, vacuolation of the adrenal cortex and fibrocystomas in the mammary gland</p> <p>No treatment-related increase in tumours incidence</p> <p>In all animals, benign tumours (adenoma of the pituitary gland, fibroadenomas and multiple fibroadenomas of the mammary</p>	Anonymous 34, 1990	/

CLH REPORT FOR NITROMETHANE

		glands), not treatment-related 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 <i>et al.</i> , 1982	/
No guideline, carcinogenicity study	1-NP	No increase of tumour incidence	Fiala <i>et al.</i> , 1987	/
No guideline, Test for chemical carcinogens	1-NP	No increase in tumour incidence	Hadidian <i>et al.</i> , 1968	/

Table 64: 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
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 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
	Liver Hepatocellular adenoma or carcinoma High background incidence		Yes	Yes	Only in females	No	Inhalation	
NITROETHANE								
Rat (Long-Evans)	Pituitary adenoma Increase similar in	No	No	/	Both	/	Inhalation	/

CLH REPORT FOR NITROMETHANE

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
	controls No data on background incidence							
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	/

10.9.2 Comparison with the CLP criteria

CLP criteria cat. 1	CLP criteria cat. 2
<p>Known or presumed human carcinogens</p> <p>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:</p> <p>Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or</p> <p>Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.</p> <p>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:</p> <ul style="list-style-type: none"> – 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). <p>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</p>	<p>Suspected human carcinogens</p> <p>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.</p>

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 malignant 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 65: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
NITROMETHANE			
13-week repeated dose inhalation toxicity study No guideline GLP-compliant Fischer 344 Rat 10/sex/dose 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: 6h12min/d, 5 d/w, for 13 w	Mortality: / BW: Significant 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 Organ weights: no changes Sexual function and fertility: Reproductive data: no sign. change in the estrous cycle length Sign. decrease in sperm motility at 750 and 1500 ppm	NTP, 1997
13-week repeated dose inhalation toxicity study No guideline GLP-compliant B6C3F1 mice 10/sex/dose 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: 6h12min/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			

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
<p>13-week inhalation toxicity study</p> <p>Similar to OECD TG 413</p> <p>Mainly GLP</p> <p>Mouse</p> <p>B6C3F1</p> <p>Male/female</p> <p>15/sex/dose</p> <p>Reliability 2 (according to the registration dossier)</p>	<p>Nitroethane</p> <p>Purity: > 97 %</p> <p>Inhalation</p> <p>6 h/d, 5 d/wk, 13 w</p> <p>Doses: 0, 100, 350, 1000 ppm equivalent to 0, 0.3, 1.0, 3.0 mg/L, resp.</p>	<p>Parental toxicity:</p> <p>No effect on BW, food consumption, clinical signs</p> <p>At 1000 ppm: Effects seen in the salivary glands, liver, and olfactory nasal epithelium</p> <p>At 350 ppm: Effects seen in liver, salivary glands and nasal turbinates and MetHb levels were affected</p> <p>At 100 ppm: Minimal changes reported (only in nasal turbinates and transiently in salivary gland epithelium)</p> <p>Sexual function and fertility:</p> <p>Sperm parameters not evaluated</p> <p>At 1000 ppm:</p> <p>Effects seen in the testes as sign 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), 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)</p> <p>In females at terminal kill: primary benign teratoma in ovary (1/5), very slight focal muscularis acute inflam. in cervix (1/5)</p> <p>At 350 ppm: In testis, significant increase of relative testicular weight</p>	<p>Anonymous 26, 1982</p>
<p>13-week inhalation toxicity study</p> <p>Similar to OECD TG 413</p> <p>Mainly GLP</p> <p>Rat</p>	<p>Nitroethane</p> <p>Purity: >97 %</p> <p>Inhalation</p> <p>6 h/d, 5 d/wk, 13 w</p> <p>Doses: 0, 100, 350, 1000</p>	<p>Parental toxicity:</p> <p>Statistically sign. 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)</p> <p>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</p>	<p>Anonymous 26, 1982</p>

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
F344 15/sex/dose Reliability 2 (according to the registration dossier)	ppm equivalent to 0, 0.3, 1.0, 3.0 mg/L, resp	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 sign way, in the 350 and 1000 ppm groups, in comparison with the controls.	
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 occurred.	Beliles <i>et al.</i> , 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 occurred.	Heicklen <i>et al.</i> , 1979
1-NITROPROPANE			
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Rat (SD) (CrI: 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 pre-mating period, during mating for both sexes and until gestation	Parental Mortality: none Clinical signs: no effects observed BW: in males only: a trend to decrease was noted and was significantly lower at the highest dose at D7 of the pre-mating period Organ weight: in males at highest dose: signif. lower FBW and signif. 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) <i>Litter size: lower at the highest dose (not signif. however outside the range of</i>	Anonymous 37, 2003

CLH REPORT FOR NITROMETHANE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	day 19 for females	<i>HCD)</i> <i>Pup BW: significantly higher at 100 ppm in both sexes at lactation day 1 and 4 (within HCD range)</i>	

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 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 66). 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 Hb concentration at all time points in all animals exposed to 375, 750 and 1500 ppm and at several time points at 94 and 188 ppm). No modifications were reported in organ weights.

Table 66: 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 67: 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	338 ± 7	341 ± 4	331 ± 4	299 ± 11**

CLH REPORT FOR NITROMETHANE

	termination				
	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; *= 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 (approximately 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 68: Organ weights

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

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 interpret 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 69: 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 70: Estrous cycle length

Exposure level (ppm)	0	375	750	1500
Length (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 106) was observed, as well as an increase in methemoglobin levels (associated with cyanosis), 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 (**Error! Reference source not found.** 107).

- Dull, dark red eyes were very pronounced in the 1000 ppm group (appeared after the first exposure and thereafter), while it 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 occurred 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 107), 15 hours after the last exposure (Part A of Table 107). 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 107).

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 107).

At terminal kill, a time-sequenced analyse (Part D of Table 107Table 108) 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 108)

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

Table 71: 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 72: 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 73: 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 74: 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 75: 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 76: Histopathological observations

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 epididymis 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

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 ay 7 of the pre-mating period (see Table 77). These change were not observed in females (see Table 78).

Table 77: 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 78: 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 (Ctrl: 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 80).

Table 79: 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 80: Incidence of nasal tissue degeneration

Dose level (in ppm)		Males				Females			
		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)	V. S.	0	0	0	1	0	0	0	5
	S.	0	0	0	1	0	0	0	2
Degeneration of the olf. epith. with inflammation (focal)	V. S.	0	0	0	0	0	0	2	0
Degeneration of the olf. epith. with inflammation (multifocal)	S.	0	0	0	0	0	0	0	2
Chronic inflammation of the epith. (squamous cell) (focal)	V. S.	0	0	0	0	2	1	0	0
	S.	0	0	0	0	0	0	0	1
Chronic inflammation of the epith. (squamous cell) (multifocal)	V.S.	0	0	0	0	1	1	1	2
	S.	0	0	0	1	0	0	2	1

S. = slight, V.S.= very slight

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).

10.10.3 Comparison with the CLP criteria

CLP criteria Category 1	CLP criteria Category 2
<p>“known or presumed human reproductive toxicant</p> <p>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).”</p>	<p>“Suspected human reproductive toxicant</p> <p>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.</p> <p>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.”</p>

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

➤ Sperm parameters:

Sperm was examined in two studies performed with nitromethane. As observed in Table 81, 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 (Anonymous 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.

➤ Male reproductive organ:

As observed in Table 81, male reproductive organ exhibited variation in different studies. Some of them were significant.

Table 81: 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 (Anonymous 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 significantly 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 (Anonymous 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 82: 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 83: 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
NITROMETHANE			
<p>Prenatal Developmental Toxicity Study</p> <p>Rat (Wistar)</p> <p>24 females/group (2 females mated with 1 male)</p> <p>OECD TG 414</p> <p>GLP</p> <p>Reliability 1 (according to the registration dossier)</p> <p>Deviations: identification of males via a subcutaneous transponder and not a mark on the tail, variation of the relative humidity from 44.9 to 65 % and no use of the surplus animals for training purpose.</p>	<p>Nitromethane</p> <p>Purity: > 99 %</p> <p>Inhalation (vapours)</p> <p>Doses: 0, 300, 600 and 1200 ppm (\pm 0, 0.75, 1.50 and 3 mg/L, resp.y)</p> <p>Duration of exposure: 6 h/d, from GD 6 to 20</p>	<p>Actual concentrations in chamber: 303, 601 and 1178 ppm (similar to 0.75, 1.50 and 2.99 mg/L, resp.)</p> <p>Maternal toxicity:</p> <p>Mortality: /</p> <p>Clinical sign: no abnormal change reported</p> <p>BW: sign. decreased at days 18 and 21 at 1200 ppm</p> <p>BWG: sign. decreased from D15 to D21</p> <p>Organ weight: sign. decreased relative ovaries, relative liver, absolute and relative kidney weights at 1200 ppm</p> <p>Food consumption: stat. sign. decreased between days 6-9 and 18-21 at 1200 ppm</p> <p>Parental necropsy: no treatment-related macroscopic modification observed</p> <p>Developmental effects:</p> <p>Post-implantation loss: stat. sign. increase in the % of late resorptions and in % of post-implantation loss at 1200 ppm</p> <p>Nb of foetuses: stat. sign. decrease in the mean number of foetuses per dam at 1200 ppm</p> <p>Gravid uterus weight: stat. sign. decreased gravid uterus weight at 1200 ppm</p> <p>Pup bw: at 1200 ppm stat. sign. decreased BW at birth, in both sexes</p> <p>Developmental abnormalities (including malformations): stat. sign. increase in the % of pale foetuses per litter, in the % of</p>	<p>Anonymous 36, 2017</p>

CLH REPORT FOR NITROMETHANE

		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 <i>et al.</i> , 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 occurred.	Beliles <i>et al.</i> , 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 occurred.	Heicklen <i>et al.</i> , 1979
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 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 pre-mating 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 pre-mating 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

CLH REPORT FOR NITROMETHANE

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 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 (approximately 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 87).

No abnormal change was reported in clinical signs.

Table 84: BW at the start of the study in females and evolution during gestation

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 85: 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 86: 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 87: 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 fetuses 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 88: 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 89). 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 90 and Table 91. Hematological parameters were not monitored in dams, nor in foetuses.

Table 89: Foetal body weights (g)

Doses (ppm)	0	300	600	1200
N	17	19	20	17
Female	4.80 ± 0.31	4.91 ± 0.25	4.76 ± 0.34	3.65** ± 0.37
N	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 middle dose groups. No visceral malformation were observed in any dose group.

Table 90: 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 91: 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 level 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 $\frac{1}{2}$ 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-histidine 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

/

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 \pm 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.

Authors attributed these effects to maternal toxicity and/or stress induced by nasal irritation. No more information that could explain this reduction is available in the full study report (e.g. on possible resorption or else). As the study states that no mortality was reported and neither behavior, nor demeanor of any animals at any exposure level was impacted throughout the study by the treatment. Furthermore, no treatment-related or significant clinical observation was noted. Feed consumption, BW and BWG was not affected throughout the gestation or lactation periods, at any dose levels.

Considering these observations, the DS is of the opinion that litter size reduction at the highest dose may be caused by the treatment. 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-implantation 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. Furthermore, maternal toxicity does not explain either the decreased litter size at the highest dose since no clinical sign was observed in mothers and body weight and organ weights were unaffected by the treatment.

The survival index and sex ratio were unaffected (see Table 92). 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 93). Variations and malformations were not examined in the study as well as the physical landmarks.

Table 92: 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 93: Pup body weight data (in g)

Dose level (in ppm)	Males					Females				
	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

10.10.6 Comparison with the CLP criteria

CLP criteria Category 1	CLP criteria Category 2
“Known or presumed human reproductive toxicant Substances are classified in category 1 for	“Suspected human reproductive toxicant Substances are classified in category 2 for

<p>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).”</p> <p>Category 1A: Known human reproductive toxicant The classification of a substance in this Category 1A is largely based on evidence from humans.</p> <p>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.</p>	<p>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.</p> <p>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.”</p>
---	---

Table 94: 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 (Anonymous 37, 2003)	5.43, 7.98, 3.97 and 7.06 %	14.0, 14.3, 15.1 and 11.9	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 approximately 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 rest 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 95: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
<p>Combined repeated dose toxicity with the reproduction/developmental toxicity screening test</p> <p>Rat (SD) 12/sex/dose OECD TG 422 GLP Reliability 1 (according to the registration dossier)</p>	<p>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: 14 d of pre-mating period, during mating for both sexes and until gestation day 19 for females</p>	<p>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 pre-mating period Pups Pup BW: sign. higher at 100 ppm in both sexes at lactation day 1 and 4 (within HCD range)</p>	<p>Anonymous 37, 2003</p>

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 (approximately 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 96). At the highest dose, a significant higher pup body weight was noted in both sexes at D1 and D4 (see Table 97).

Table 96: 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 97: 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 sacrificed 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, foetuses were observed until the lactation day 4. The survival index was unaffected and the pups body weight increased at the highest dose (within the HCD range).

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 98: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
NITROMETHANE			
<p>16-day repeated dose toxicity study</p> <p>Rat (F344)</p> <p>5/sex/dose</p> <p>Non-GLP</p> <p>No guideline</p> <p>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)</p>	<p>Nitromethane</p> <p>Purity: > 98 %</p> <p>Inhalation (vapours)</p> <p>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.).</p> <p>Duration: 16 days, 6 h/d for 5 d/w</p>	<p><u>1500 ppm (3.750 mg/L)</u></p> <p>Sign. decreased BWG in males compared to controls</p> <p><i>Nervous system:</i> Sciatic nerve degeneration in 5/5 males and 5/5 females</p> <p><i>Respiratory tract:</i> Degeneration of the olf. epith. in 5/5 males and 5/5 females</p> <p><u>750 ppm (1.880 mg/L)</u></p> <p><i>Nervous system:</i> Sciatic nerve degeneration in 5/5 males and 5/5 females</p> <p><i>Respiratory tract:</i> Degeneration of the olf. epith. in 5/5 males and 5/5 females</p> <p><u>375 ppm (0.938 mg/L)</u></p> <p><i>Nervous system:</i> Sciatic nerve degeneration in 5/5 males and 4/5 females</p> <p><i>Respiratory tract:</i> Degeneration of the olf. epith. in 5/5 males and 5/5 females</p> <p><u>188 ppm (0.47 mg/L) and lower</u></p> <p>No treatment-related effect in males and females</p> <p>LOAEC: 375 ppm</p>	NTP, 1997

CLH REPORT FOR NITROMETHANE

<p>16-day repeated dose toxicity study</p> <p>Mouse (B6C3F1)</p> <p>10/sex/dose</p> <p>Non-GLP</p> <p>No guideline</p> <p>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)</p>	<p>Nitromethane</p> <p>Purity: > 98 %</p> <p>Inhalation (vapours)</p> <p>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.).</p> <p>Duration: 16 days, 6 h/d for 5 d/w</p>	<p><u>1500 ppm (3.750 mg/L)</u></p> <p><i>Respiratory tract:</i> Degeneration of the olf. epith. in 10/10 males and 10/10 females;</p> <p>Increased absolute and relative liver weight in males and females</p> <p><u>750 ppm (1.880 mg/L)</u></p> <p><i>Respiratory tract:</i> Degeneration of the olf. epith. in 10/10 males and 10/10 females;</p> <p>Increased absolute and relative liver weight in males and females</p> <p><u>375 ppm (0.938 mg/L)</u></p> <p><i>Respiratory tract:</i> Degeneration of the olf. epith. in 10/10 males and 10/10 females;</p> <p>Increased absolute and relative liver weight in females. Increased relative liver weight in males.</p> <p><u>188 ppm (0.47 mg/L)</u></p> <p>Increased absolute and relative liver weight in females</p> <p><u>94 ppm (0.235 mg/L)</u></p> <p>Increased absolute and relative liver weight in females</p> <p>LOAEC: 375 ppm</p>	<p>NTP, 1997</p>
<p>13-week repeated dose inhalation toxicity study</p> <p>Rat (Fischer 344)</p> <p>10/sex/dose</p> <p>Similar to OECD TG 413</p> <p>GLP-compliance not specified</p> <p>Reliability 1 (according to the registration dossier)</p>	<p>Nitromethane</p> <p>Purity: > 98 %</p> <p>Inhalation (vapours)</p> <p>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.).</p> <p>Duration: 13 weeks, 6 h/d for 5 d/w</p>	<p><u>1500 ppm (3.750 mg/L)</u></p> <p>Decreased FBW (-12 %) and BWG (-19 %) in males compared to controls</p> <p><i>Nervous system:</i> 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</p> <p>Startle response amplitude decreased in males and females</p> <p><i>Respiratory tract:</i> Degeneration of the olf. epith. in 10/10 males and 10/10 females + hyaline droplets in 8/10 males and 10/10</p>	<p>NTP, 1997</p>

CLH REPORT FOR NITROMETHANE

		<p>females</p> <p>Bone marrow hyperplasia in 10/10 males and 10/10 females</p> <p>Goblet cells hyperplasia in 10/10 males and 10/10 females</p> <p>Sign. decrease in T3, thyroxine and free thyroxine in both sexes at day 23</p> <p>Sign. increase in erythrocytes and MetHb levels at week 13</p> <p>Sign. decrease in the weight of left cauda, epididymis and testis</p> <p><u>750 ppm (1.880 mg/L)</u></p> <p><i>Nervous system:</i> Sciatic nerve and spinal cord degeneration in 10/10 males and 10/10 females</p> <p>Startle response amplitude decreased in males and females</p> <p><i>Respiratory tract:</i> Degeneration of the olf. epith. 10/10 males and 10/10 females; hyaline droplets in 4/10 females</p> <p>Bone marrow hyperplasia in 9/10 males and 7/10 females</p> <p>Significant increase in erythrocytes and MetHb levels at week 13</p> <p><u>375 ppm (0.938 mg/L)</u></p> <p><i>Nervous system:</i> Sciatic nerve (5/10 males and 8/10 females) and spinal cord (9/10 males) degeneration</p> <p>Startle response amplitude decreased in males</p> <p><i>Respiratory tract:</i> Degeneration of the olf. epith. in 9/10 males and 10/10 females</p> <p>Bone marrow hyperplasia in 6/10 females</p> <p>OSign. increase in erythrocytes and MetHb levels at week 13</p> <p><u>188 ppm (0.47 mg/L) and lower</u></p> <p>Sign. increase in erythrocytes and MetHb levels at week 13</p> <p>LOAEC (systemic, male/female): 188 ppm (0.470 mg/L) based on disturbance of hematological parameters</p> <p>NOAEC (systemic, male/female): 94 ppm (0.235 mg/L)</p> <p>LOAEC (local, male/female): 375 ppm (0.938 mg/L) for the upper respiratory tract</p> <p>NOAEC (local, male/female): 188 ppm (0.470 mg/L)</p>	<p>108</p>
--	--	--	------------

CLH REPORT FOR NITROMETHANE

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

CLH REPORT FOR NITROMETHANE

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

CLH REPORT FOR NITROMETHANE

<p>Sub-chronic repeated dose 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)</p>	<p>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</p>	<p><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. <i>Lung</i> : 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</p>	<p>Lewis <i>et al.</i>, 1977</p>
<p>Sub-chronic repeated-dose toxicity study Rat (albino) 10 males/dose Non-guideline Non-GLP Reliability 4 (according to the registration dossier)</p>	<p>Nitromethane Purity: unknown Oral (drinking water) Doses: 0, 0.1, 0.25 % (0.5, 1 and 2 %) Duration: 15 weeks</p>	<p>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 <i>Liver</i>: less stained and more granular liver cell cytoplasm, more lymphocytes in the periportal zone in 6/7 surviving animals <i>Spleen</i>: prominent Malpighian corpuscles in 2/7 surviving animals <u>0.1 % (150 mg/kg bw/d)</u> 4/10 animals died</p>	<p>Weatherby <i>et al.</i>, 1955</p>

CLH REPORT FOR NITROMETHANE

		<p>Decreased body weight in surviving animals</p> <p><i>Liver</i>: enlarged hepatic cells in 2/6 surviving animals</p> <p>LOAEL: 0.1 % (150 mg/kg bw/d)</p> <p>No NOAEL</p>	
<p>2-year repeated dose inhalation study</p> <p>Equivalent or similar to OECD TG 451</p> <p>GLP-compliant</p> <p>2 years</p> <p>Mice (B6C3F1)</p> <p>50/sex/group</p> <p>Reliability 1 (according to the registration dossier)</p>	<p>Nitromethane</p> <p>Purity: > 99 %</p> <p>Impurities: 0.25 % nitroethane, 0.03 % 2-nitropropane</p> <p>Inhalation</p> <p>6 h/d, 5 d/week</p> <p>Doses: 0, 188, 375, 750 ppm (approx. equivalent to 0, 0.47, 0.94 and 1.87 mg/L, resp.)</p>	<p><i>Mortality</i>: 38, 28, 40 and 42 % of M and 50, 44, 48 and 28 % of F exposed to 0, 188, 375 and 750 ppm, resp.</p> <p><i>Clinical sign</i>: in the eyes, swelling and exophthalmos coincident with harderian gland tumours, in both sexes</p> <p><i>BWG</i>: no effects in males, slightly increased BW in females during the study but similar to controls at study termination</p> <p><i>Organ weights</i>: no data</p> <p><i>Histopathology</i>:</p> <ul style="list-style-type: none"> - sign. increased incidence olf. epith. degeneration in both sexes, in all treated groups - sign. increase in olf. epith. metaplasia in both sexes at 375 and 750 ppm - sign. increase in resp. epith. hyaline degeneration in all treated groups in females and at the middle and high doses in males. <p><i>Neoplastic effects</i></p> <p>Harderian gland: Male and female:</p> <p>Adenoma (%):</p> <p style="margin-left: 20px;">M: 9/49 (18), 10/50 (20), 19/50 (38), 32/49 (65)</p> <p style="margin-left: 20px;">F: 5/49 (10), 7/49 (14), 16/50 (32), 19/50 (38)</p> <p>Carcinoma (%):</p> <p style="margin-left: 20px;">M: 1/49 (2), 1/50 (2), 6/50 (12), 5/49 (10)</p> <p style="margin-left: 20px;">F: 1/49 (2), 2/49 (4), 4/50 (8), 3/50 (6)</p> <p>Adenoma or carcinoma (%):</p> <p style="margin-left: 20px;">M: 10/49 (20), 11/50 (22), 25/50 (50), 37/50 (74)</p>	<p>NTP, 1997</p>

CLH REPORT FOR NITROMETHANE

		<p>F: 6/49 (12), 9/49 (18), 20/50 (40), 21/50 (42)</p> <p>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.</p> <p>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)</p>	
NITROETHANE			
<p>13-week repeated dose inhalation toxicity study Rat Fischer 344 15/sex/dose OECD TG 413 GLP: Study was initiated prior to GLP and completed with GLP</p>	<p>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</p>	<p><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</p>	<p>Anonymous 26, 1982</p>

CLH REPORT FOR NITROMETHANE

<p>Reliability 2 (according to the registration dossier) Deviation: food consumption not assessed</p>	<p>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</p>	<p>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</p>	
<p>13-week repeated dose inhalation toxicity study Mice (B6C3F1) 5/sex/dose OECD TG 413 Deviations: yes GLP: Study was initiated prior to GLP and completed with GLP Reliability 1 (according to the registration dossier)</p>	<p>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</p>	<p><u>At 1000 ppm (3 mg/L):</u> Increased MetHb concentration including the increased presence of reticulocytes and Heinz bodies Moderate degeneration of the olf. 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 <u>At 350 ppm (1 mg/L):</u> Less extensive toxicity, only MetHb, nasal turbinates and liver affected <u>At 100 ppm (0.3 mg/L):</u> Minimal changes in nasal turbinates (females only) and transient effects (at 29 days not 13 weeks) on salivary glands LOAEC: 100 ppm</p>	<p>Anonymous 26, 1982</p>

CLH REPORT FOR NITROMETHANE

<p>Range-finding study for 13-weeks repeated dose inhalation toxicity study</p> <p>Rat (Fischer 344)</p> <p>5/sex/dose</p> <p>GLP: Study was initiated prior to GLP and completed with GLP</p>	<p>Nitroethane</p> <p>Purity: unknown</p> <p>Inhalation: vapours</p> <p>Doses: 0, 350, 1000, 2000 or 4000 ppm (equivalent to 0, 1.0, 3.0, 6.0 or 12 mg/L, resp.)</p> <p>Exposure period: 4 d</p>	<p>Please refer to chapter 10.3 (Inhalation acute toxicity study, 4-day study in rats)</p> <p>All animals died at the highest dose: probable cause: hypoxia secondary to methemoglobinemia</p> <p>Specific toxicity from 350 ppm:</p> <ul style="list-style-type: none"> - cyanosis, a manifestation of the MetHb effect determined in the 13-week study - hyperemia of the nasal turbinates <p>LOAEC: 350 ppm</p>	<p>Anonymous 26, 1982</p>
<p>Chronic inhalation toxicity study</p> <p>2 years</p> <p>Similar to OECD TG 453</p> <p>GLP compliant: nt specified</p> <p>Rat (Long-Evans)</p> <p>40/sex/group (control & 100 ppm)</p> <p>41 males & 39 females (200 ppm)</p> <p>Reliability 2 (according to the registration dossier)</p> <p>Major deviations:</p> <ul style="list-style-type: none"> - only 2 doses tested - 40 animals / group - some tissues were not examined microscopically (parathyroid, caecum, rectum, bone marrow,...) 	<p>Nitroethane</p> <p>Purity: 97.92 %</p> <p>Impurities: nitromethane 0.01 % and 2-nitropropane 2.07 %</p> <p>Inhalation</p> <p>7 h/d, 5 d/w</p> <p>Conc.: 0, 100, 200 ppm (corresp. approx. to 0, 0.31 and 0.61 mg/L, resp.)</p>	<p><i>Mortality:</i> no treatment-related effect</p> <p><i>BW:</i> sign. ↓ at 100 ppm in males and at 200 ppm in females</p> <p><i>Clinical chemistry:</i> slight but sign. ↑ of total protein and BUN in females exposed to 200 ppm</p> <p><i>Hematology:</i> No effects observed. MetHb level not reported.</p> <p><i>Organ weights (brain, liver, kidneys, lungs, heart):</i> no treatment-related effect</p> <p><i>Histopathology:</i> no effect</p> <p><i>Neoplastic effects:</i></p> <ul style="list-style-type: none"> 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 	<p>Anonymous 35, 1986</p>
1-NITROPROPANE			
<p>Short-term repeated dose toxicity study</p> <p>Rat (SD)</p>	<p>1-nitropropane</p> <p>Purity: > 98.5 %</p>	<p><u>100 mg/kg bw/d</u></p> <p><i>Males</i></p> <p>1 male killed in extremis at D27 (necropsy: dark kidneys,</p>	<p>Anonymous 38, 1996</p>

CLH REPORT FOR NITROMETHANE

<p>5/sex/dose Japanese guideline GLP Reliability 1 (according to the registration dossier)</p>	<p>Oral (gavage) Doses: 0, 10, 30 and 100 mg/kg bw/d + 2 additional group 0 and 100 mg/kg bw/d (recovery group) Duration of exposure: 28 d Recovery period: 14 d</p>	<p>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) <i>Females</i> 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> <i>Males</i> No treatment-related effect in males <i>Females</i> 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</p>	
<p>Range-finding study of the 28-day repeated dose toxicity study Rat (SD) 3/sex/dose</p>	<p>1-nitropropane Oral (gavage) Doses: 0, 10, 50, 150 and 250 mg/kg bw/d Duration of exposure: up to 14 d</p>	<p><u>250 mg/kg bw/d</u> 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, dehydration, emaciation Gross pathology findings: pale kidneys, pale liver, pale adrenals, epithelial sloughing of the non-glandular stomach <u>150 mg/kg bw/d</u></p>	<p>Anonymous 38, 1996</p>

CLH REPORT FOR NITROMETHANE

		<p>Mortality: one male killed in extremis on D7</p> <p>Clinical signs: ataxia, body tremors, pallor of extremities, loss of righting reflex</p> <p>Gross pathology findings: pale kidneys, epithelial sloughing of the non-glandular stomach</p> <p><u>50 mg/kg bw/d & 10 mg/kg bw/d</u></p> <p>No treatment-related effect</p> <p>NOAEL: 50 mg/kg bw/d</p> <p>LOAEL: 150 mg/kg bw/d</p>	
<p>Combined repeated dose toxicity with the reproduction/developmental toxicity screening test</p> <p>Rat (SD)</p> <p>12/sex/dose</p> <p>OECD TG 422</p> <p>GLP</p> <p>Reliability 1 (according to the registration dossier)</p> <p><i>For males, +- 28 d exposure: Guidance value range for warranting classification as cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d</i></p> <p><i>cat. 1: C ≤ 0.6 mg/L/6 h/d</i></p> <p><i>for females: +- 45 d exposure, Guidance value range for warranting classification as cat. 2: : 0.4 < C ≤ 2 mg/L/6 h/d</i></p> <p><i>cat. 1: C ≤ 0.4 mg/L/6 h/d</i></p>	<p>1-nitropropane</p> <p>Purity: 99.69 %</p> <p>Inhalation (vapours)</p> <p>Doses: 0, 25, 50 and 100 ppm (corresponding to approx. 0, 0.092, 0.184 and 0.369 mg/L)</p> <p>Duration of exposure: 6 h/d, 14 d of pre-mating period, during mating for both sexes and until gestation day 19 for females</p> <p>6 h/d, 7 d/w</p>	<p>Mortality: /</p> <p>Clinical signs: no effects observed</p> <p><u>At 100 ppm (0.369 mg/L):</u></p> <p>BW: tendency to ↓ in males (stat. sign. at day 7 of the pre-mating period)</p> <p>Organ weight: in males: ↓ FBW and ↑ relative brain weight and relative testes weights</p> <p>Histopathology: multifocal degeneration of the olf. epith. (only in 7 females); associated inflammation in 2 females</p> <p><u>At 50 ppm (0.184 mg/L):</u></p> <p>Histopathology: in females nasal tissue: inflammation and degeneration of the olf. epith. in 2 animals</p> <p><u>At 25 ppm (0.092 mg/L):</u></p> <p>No treatment-related effects</p> <p>NOAEC: 25 ppm (0.184 mg/L)</p> <p>LOAEC: 50 ppm (0.369 mg/L)</p>	<p>Anonymous 37, 2003</p>

CLH REPORT FOR NITROMETHANE

Table 99: 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 <i>et al.</i> , 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 methemoglobinemia, 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 <i>et al.</i> , 1994

CLH REPORT FOR NITROMETHANE

<p>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</p>	<p>Nitroethane Purity: unknown 4.5, 6.7 or 9.0 mmol/kg IP</p>	<p>Reporting deficiencies (doses not clearly stated for example)</p>	<p>No sign. increase in SDH, ALT or AST activity. No significant abnormalities in livers of mice exposed to 9 mmol/kg</p>	<p>Dayal R <i>et al.</i>, 1989</p>
--	---	---	--	------------------------------------

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

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. At 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 cytoplasm 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 6h + 12 minutes 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 100: 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-d study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 1.2 mg/L/d for Cat. 1 and $1.2 \leq C \leq 6$ mg/L/d for Cat. 2, respectively. The dossier submitter considers therefore this 16-d 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 effects 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 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/6h/day, respectively) nitromethane by inhalation for 6h 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-d study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 1.2 mg/L/d for Cat. 1 and $1.2 \leq C \leq 6$ mg/L/d for Cat. 2, respectively. The dossier submitter considers therefore this 16-d repeated dose toxicity study as relevant for STOT RE classification.

In a 13-week inhalation repeated dose 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 101: 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 Htc 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 102: 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 103: 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 $0.2 \leq C \leq 1$ 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 pp, respectively). No kidney, liver or lung lesions were observed in exposed mice.

Table 104: 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 $0.2 \leq C \leq 1$ 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 105: 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 $0.2 \leq C \leq 1$ 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 areas of mild to severe haemorrhage and congestion of the alveolar area and duct walls. Edema and sometimes necrosis were seen in the congested 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 $0.2 \leq C \leq 1$ 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 $0.025 \leq C \leq 0.125$ 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 equivalent 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). Coincidentally 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 $0.025 \leq C \leq 0.125$ 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 weights, 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 cyanosis), 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.

CLH REPORT FOR NITROMETHANE

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 106: Rat Body weights in a 13 weeks inhalation toxicity study (in g)

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

CLH REPORT FOR NITROMETHANE

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 methemoglobinemia.

- Dull, dark red eyes were very pronounced in the 1000 ppm group (appeared after the first exposure and thereafter), while it 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 unkempt 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 occurred 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 107). 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 107).

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 107).

At terminal kill, a time-sequenced analyse (Part D of Table 107) was performed less than 30 min after exposure, 4 and 19 h after exposure in rats. 19-h after exposure, methemoglobinemia was similar in control, 100 and 350 ppm groups. The level was however significantly increased at 1000 ppm.

Table 107: Methemoglobinemia

Males					Females			
0	100	350	1000	Dose levels	0	10	350	1000
A: 15 h after the 20 th 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 29 th exposure, in females only								
-	-	-	-	N	5	-	-	5
-	-	-	-	MetHb	0.6±0.5	-	-	57.4*±5.2
C: immediately after the 30 th exposure								

CLH REPORT FOR NITROMETHANE

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 64 th (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: 4 h 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: 19 h 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 108: 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 (x10⁶/mm³); Hb= Hemoglobin (g/100ml); WBC= White blood cells (x10³/mm³); Reticulocytes (%); Heinz bodies (%); *p<0.05

Histological assessment is described in Table 109 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 109: Histopathological assessment

Dose levels (ppm)	Males				Females			
	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

CLH REPORT FOR NITROMETHANE

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 multifoc 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

CLH REPORT FOR NITROMETHANE

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

CLH REPORT FOR NITROMETHANE

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 weights, 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 110: Haematological parameters

Males				Exposure (ppm)	Females			
0	100	350	1000		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

CLH REPORT FOR NITROMETHANE

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 (x10⁶/mm³); Hb= Hemoglobin (g/100ml); WBC= White blood cells (x10³/mm³); 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 111: Methemoglobinemia

Males				Dose levels	Females			
0	100	350	1000		0	10	350	1000
5	5	5	5	N	5	5	5	5
Immediately after the 64 th (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
4 h after last exposure								
Not det.	Not det.	Not det.	7.4 ± 2.6	MetHb	Not det.	Not det.	Not det.	10.4 ± 2.9
19 h after last exposure								
0.8 ± 0.7	0.8 ± 0.4	1.3 ± 1.0	0.9 ± 0.4	MetHb	1.1 ± 0.3	0.9 ± 0.6	1.3 ± 0.4	2.4* ± 0.8

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

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 112: 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); ALP= alkaline phosphatase (mU/mL); P= phosphorus (mg/100mL)

Prior to the terminal kills (92 days), no effects were seen on SGPT, AP, glucose, phosphorus and calcium levels 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 113: 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 <i>already punctured the day before</i> 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

CLH REPORT FOR NITROMETHANE

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 <i>never bled</i> before)								
34±5	29±2	20*±2	27±6	BUN	26±4	21±3	19*±2	20*±3
45±6	36±5	38±4	39±7	ALP	54±8	60±7	55±6	63±12
10.7±2.0	8.3±0.3	9.3±1.9	9.4±1.0	P	8.2±0.6	7.3±1.2	8.0±0.9	8.4±1.1
10.5±0.6	11.2±0.8	9.9±0.3	10.0±0.2	Ca	10.2±0.2	10.0±0.5	9.8±0.2	9.6*±0.1

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

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 weights 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 114: Organ weights

Dose levels (ppm)	Males				Females			
	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

CLH REPORT FOR NITROMETHANE

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 epididymis 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 115: Histopathological modifications

Dose levels (ppm)	Males				Females			
	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 tissues 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

CLH REPORT FOR NITROMETHANE

Moderate decrease in eosinophilia	0	0	0	0	0	0	5	4
Mediastinal tissue: N tissues examined	5	4	2	4	3	5	2	5
Multifocal monocl.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 homogeneity	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

CLH REPORT FOR NITROMETHANE

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 acute 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 $0.025 \leq C \leq 0.125$ 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 116). A rebound in methemoglobin increased its level up to 53 % 23 hours after ingestion.

Table 116: 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.

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).

1 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 117). 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 117: Body weight data (in g)

Dose level (in mg/kg bw/d)	Main groups				Recovery groups	
	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 and high dose groups. The same tendency was noted in females as well with a dose-dependent increase in methemoglobin in the main groups. Furthermore, higher clotting time was observed in females and lower platelet count was noted in males (see Table 118).

Table 118: 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 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). 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 90-d 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 histopathological effects 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 > 30 and ≤ 300 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 significantly affected in females (see Table 77 and Table 78). At necropsy, organ weights were examined and revealed few significant changes (see Table 79). 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/100g at 0, 25, 50 and 100ppm, respectively) and relative testes weight (0.867, 0.902, 0.846 and 0.965* g/100g 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 80).

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 approximately 28 days, the guidance values range for warranting classification as cat. 2 is $0.6 < C \leq 3$ mg/L/6h/d and as cat. 1: $C \leq 0.6$ mg/L/6h/d. For females exposed approximately for 45 days, the guidance values range for warranting classification as cat. 2 is $0.4 < C \leq 2$ mg/L/6h/d and as cat. 1: $C \leq 0.4$ 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

/

Table 119: 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	Degeneration and	Male: min. 28 D	Male: ± 0.12 mg/L	STOT RE Cat. 1

CLH REPORT FOR NITROMETHANE

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
toxicity study with the reproduction/developmental toxicity screening test in Rat Inhalation route 1-nitropropane Anonymous 37, 2003	inflammation of the olf. epith. at 100 ppm corresp. approx to 0.369 mg/L	Female: ± 45 D	Female: ± 0.18 mg/L	As ≤ 0.2 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 <i>et al.</i> , 1977	No sign. effect in the repirsatory tract (however, nasal cavity not examined microscopically)	13 W	/	/
Sub-chronic repeated dose toxicity study in Rabbit Inhalation route Nitromethane Lewis <i>et al.</i> , 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

CLH REPORT FOR NITROMETHANE

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
13-week repeated dose toxicity study in Rat Inhalation route Nitroethane Anonymous 26, 1982	At interim sacrificed (± 1 month) Degeneration olf. 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)	Interim sacrifice: ± 1 month Terminal sacrifice: 92 D	± 0.3 mg/L 3.0 mg/L	STOT RE Cat. 1 after 1 month 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				

CLH REPORT FOR NITROMETHANE

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Range-finding of the 28-day repeated dose toxicity study in Rat Oral route 1-nitropropane Anonymous 38, 1996	150 mg/kg bw/d	14 D	25 mg/kg bw/d	STOT RE 2
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 performed
Sub-chronic repeated dose	1.875 mg/L	13 W	1.875 mg/L	No classification

CLH REPORT FOR NITROMETHANE

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
toxicity study in Rat Inhalation route Nitromethane Lewis <i>et al.</i> , 1977				
Sub-chronic repeated dose toxicity study in Rabbit Inhalation route Nitromethane Lewis <i>et al.</i> , 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				

CLH REPORT FOR NITROMETHANE

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Range-finding of the 28-day repeated dose toxicity study in Rat Oral route 1-nitropropane Anonymous 38, 1996	150 mg/kg bw/d	14 D	25 mg/kg bw/d	STOT RE 2
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	No effects observed	13 W	/	No classification

CLH REPORT FOR NITROMETHANE

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
toxicity study in Rat Inhalation route Nitromethane Lewis <i>et al.</i> , 1977				
Sub-chronic repeated dose toxicity study in Rabbit Inhalation route Nitromethane Lewis <i>et al.</i> , 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												
<p>“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.</p> <p>Substances are classified in category 1 for target organ toxicity (repeat exposure) on the basis of:</p> <ul style="list-style-type: none"> ▪ 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.” <p>“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”</p> <p>Table 3.9.2</p> <table border="1" data-bbox="150 1059 564 1229"> <thead> <tr> <th>Route of exposure</th> <th>Units</th> <th>Guidance value</th> </tr> </thead> <tbody> <tr> <td>Oral (rat)</td> <td>mg/kg bw/d</td> <td>C≤10</td> </tr> </tbody> </table> 	Route of exposure	Units	Guidance value	Oral (rat)	mg/kg bw/d	C≤10	<p>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.</p> <p>Substances are classified in category 2 for target toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.”</p> <p>“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”</p> <p>Table 3.9.3</p> <table border="1" data-bbox="788 893 1203 1097"> <thead> <tr> <th>Route of exposure</th> <th>Units</th> <th>Guidance value range</th> </tr> </thead> <tbody> <tr> <td>Oral (rat)</td> <td>mg/kg bw/d</td> <td>10 < C ≤ 100</td> </tr> </tbody> </table>	Route of exposure	Units	Guidance value range	Oral (rat)	mg/kg bw/d	10 < C ≤ 100
Route of exposure	Units	Guidance value											
Oral (rat)	mg/kg bw/d	C≤10											
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 120).

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 120: 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	Degeneration of the olf. epith. (multifocal) in 7 F (5 VS and 2 S)	For 28 D of	Degeneration and inflammation observed

CLH REPORT FOR NITROMETHANE

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	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 (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	exposure Cat. 2: $0.6 < C \leq 3$ mg/L/6 h/d Cat. 1: $C \leq 0.6$ mg/L/6 h/d For ± 45 D of exposure Cat. 2: $0.4 < C \leq 2$ mg/L/6 h/d Cat. 1: $C \leq 0.4$ mg/L/6 h/d	at dose relevant to classify in Cat. 1 Only very low doses 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: Rapid breathing ≥ 375 ppm: sign. increased inc. of minimal to mild degeneration of the olfactory epithelium	Cat. 2: $1.2 < C \leq 6$ mg/L/6 h/d Cat. 1: $C \leq 1.2$ 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: $1.2 < C \leq 6$ mg/L/6 h/d Cat. 1: $C \leq 1.2$ 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 olfactory 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 121: Summary data about respiratory tract in sub-chronic toxicity study

		Guidance value range for warranting classification	DS's conclusion

CLH REPORT FOR NITROMETHANE

Nitromethane			
<p>13-week repeated dose toxicity study</p> <p>Inhalation route</p> <p>Rat (F344)</p> <p>10/sex/dose</p> <p>0, 94, 188, 375, 750 and 1500 ppm (\pm 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L)</p> <p>13 w of exposure</p> <p>NTP, 1997</p>	<p>\geq 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)</p> <p>\geq 750 ppm: Hyaline droplets olf. epith. (0, /, 0, 0, 1 and 8** M and 0, 0, 0, 0, 4* and 10** F)</p>	<p>Cat. 2: $0.2 < C \leq 1$ mg/L/6 h/d</p> <p>Cat. 1: ≤ 0.2 mg/L/6 h/d</p>	<p>Sign increased inc. of degeneration olf. epith. at dose relevant to classify in Cat. 2</p>
<p>13-week repeated dose toxicity study</p> <p>Inhalation route</p> <p>Mouse (B6C3F1)</p> <p>10/sex/dose</p> <p>0, 94, 188, 375, 750 and 1500 ppm (\pm 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L)</p> <p>13 w of exposure</p> <p>NTP, 1997</p>	<p>\geq 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**)</p> <p>\geq 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**)</p>	<p>Cat. 2: $0.2 < C \leq 1$ mg/L/6 h/d</p> <p>Cat. 1: ≤ 0.2 mg/L/6 h/d</p>	<p>Increased inc. of degeneration olf. epith. at doses within the range to classify in Cat. 2</p>
<p>Sub-chronic repeated dose toxicity study</p> <p>Inhalation route</p> <p>Rat (SD) 50 M/dose</p> <p>100 and 750 ppm (\pm 0.25 and 1.875 mg/L)</p> <p>13 w of exposure</p> <p>Lewis <i>et al.</i>, 1977</p>	<p>No sign. increased incidence of effect in respiratory tract.</p> <p>However, nasal cavity not examined microscopically</p>	<p>Cat. 2 for 13-week exposure: $0.2 < C \leq 1$ mg/L/6 h/d</p>	<p>No classification</p>
<p>Sub-chronic repeated dose toxicity study</p> <p>Inhalation route</p> <p>Rabbit (NZW) 15 M/dose</p> <p>100 and 750 ppm (\pm 0.25 and 1.875 mg/L)</p> <p>13 w of exposure</p> <p>Lewis <i>et al.</i>, 1977</p>	<p>\geq 100 ppm: at the 1-month time point, modifications in the lungs as focal areas 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.</p>	<p>Cat. 2 for 13-week exposure: $0.2 < C \leq 1$ mg/L/6 h/d</p> <p>For the 1-month time point: $0.6 < C \leq 3$ mg/L/6 h/d</p>	<p>Indication of respiratory effects (after 1 month) at dose to classify in Cat. 1</p>
Nitroethane			

CLH REPORT FOR NITROMETHANE

<p>13-week repeated dose inhalation toxicity study</p> <p>Inhalation route</p> <p>Rat (F344) 15/sex/dose</p> <p>0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L)</p> <p>92 D</p> <p>Anonymous 26, 1982</p>	<p>At interim sacrifice (5 animals/sex/group examined): ± 1 month</p> <p>Slight diffuse degeneration olf. epith. in 3 M at 350 ppm and in 5 M and 5 F at 1000 ppm</p> <p>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</p> <p>At terminal kill</p> <p>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)</p> <p>+ 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)</p>	<p>For interim kill: 30-day</p> <p>Cat. 2: $0.6 < C \leq 3$ mg/L/6 h/d</p> <p>Cat. 1: ≤ 0.6 mg/L/6 h/d</p> <p>For terminal kill (90-day)</p> <p>Cat. 2: $0.2 < C \leq 1$ mg/L/6 h/d</p> <p>Cat. 1: ≤ 0.2 mg/L/6 h/d</p>	<p>Effects already observed at doses within the range to classify in Cat. 2</p>
<p>13-week repeated dose inhalation toxicity study</p> <p>Mouse (B6C3F1) 5/sex/dose</p> <p>0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L)</p> <p>93 D</p> <p>Anonymous 26, 1982</p>	<p>At interim sacrificed (5 animals/sex/group examined): ± 1 month</p> <p>Moderate olf. epith. degeneration + inflammation in 3 M and 4 F at 350 ppm and in 4 M and 5 F at 1000 ppm</p> <p>Moderate glandular hyperplasia olf. epith. in 2 M and 4 F at 350 ppm and in 4 M and 4 F at 1000 ppm</p> <p>At terminal sacrifice (5 animals/sex/group examined)</p> <p>Moderate olf. epith. degeneration + inflammation in 4 M and 5 F at 350 ppm and in 5 M and 5 F at 1000 ppm</p> <p>Moderate glandular hyperplasia olf. epith. in 4 M and 5 F at 350 ppm and in 4 M and 5 F at 1000 ppm</p>	<p>For interim kill: 30-day</p> <p>Cat. 2: $0.6 < C \leq 3$ mg/L/6 h/d</p> <p>Cat. 1: ≤ 0.6 mg/L/6 h/d</p> <p>For terminal kill (90-day)</p> <p>Cat. 2: $0.2 < C \leq 1$ mg/L/6 h/d</p> <p>Cat. 1: ≤ 0.2 mg/L/6 h/d</p>	<p>Effects already observed at doses within the range to classify in Cat. 2</p>

Chronic toxicity studies

Three chronic repeated dose toxicity studies are available (2 with nitromethane and 1 with nitroethane). As observed in Table 122, no effect was observed in 2 of these studies. While, in one of the studies performed with nitromethane, degeneration of the olfactory epithelium was observed however at dose which does not warrant a classification.

Table 122: Summary data about respiratory tract in chronic toxicity study

		Guidance value range for warranting classification	DS's conclusion
Nitromethane			
<p>2-year repeated dose inhalation toxicity study</p> <p>Inhalation route</p> <p>Rats (Fischer F344/N)</p> <p>0, 94, 188 and 375 ppm (± 0,</p>	<p>No effects</p>	<p>Cat. 2: $0.025 \leq C \leq 0.125$ mg/L/d</p> <p>Cat. 1: ≤ 0.025 mg/L/d</p>	<p>/</p>

CLH REPORT FOR NITROMETHANE

0.235, 0.47 and 0.94 mg/L) 2 y of exposure NTP, 1997			
2-year repeated dose inhalation toxicity study Inhalation route Mouse (B6C3F1) 50/sex/dose 0, 188, 375 and 750 ppm (\pm 0, 0.47, 0.94 and 1.87 mg/L) 2 y of exposure NTP, 1997	\geq 188 ppm: sign DR \uparrow olf. epith. degeneration	Cat. 2: $0.025 \leq C \leq 0.125$ 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.
Nitroethane			
Chronic inhalation toxicity study Inhalation route Rat (Long-Evans) 40/sex/dose 0, 100 and 200 ppm (\pm 0.31 and 0.61 mg/L) Anonymous 35, 1986	No effect	Cat. 2: $0.025 \leq C \leq 0.125$ 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 123, 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 123: 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 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 ataxia, body tremors, loss of righting reflex, lethargy 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 on nervous system 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 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

CLH REPORT FOR NITROMETHANE

16-day repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (\pm 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	1500 ppm: reduced activity (sciatic nerve not examined)	Cat. 2: $1.2 < C \leq 6$ mg/L/6 h/d Cat. 1: $C \leq 1.2$ mg/L/6 h/d	STOT RE 2 (but only clinical signs)
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 124: 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 (\pm 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: $0.2 < C \leq 1$ 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 (\pm 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L)	No effects observed Neurobehavioral measurement not performed	Cat. 2: $0.2 < C \leq 1$ mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	No classification

CLH REPORT FOR NITROMETHANE

13 w of exposure NTP, 1997			
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 <i>et al.</i> , 1977	No effects observed	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 <i>et al.</i> , 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	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	No classification

CLH REPORT FOR NITROMETHANE

Anonymous 26, 1982		(90-day) Cat. 2: $0.2 < C \leq 1$ mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	
--------------------	--	--	--

Chronic toxicity studies:

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

Table 125: 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 ($\pm 0, 0.235, 0.47$ and 0.94 mg/L) 2 y of exposure NTP, 1997	No effects observed	Cat. 2: $0.025 \leq C \leq 0.125$ 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 ($\pm 0, 0.47, 0.94$ and 1.87 mg/L) 2 y of exposure NTP, 1997	No effects observed	Cat. 2: $0.025 \leq C \leq 0.125$ 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: $0.025 \leq C \leq 0.125$ 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 126: 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	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

CLH REPORT FOR NITROMETHANE

D) Anonymous 38, 1996			
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Inhalation route Rat (SD) 12/sex/dose 0, 25, 50 and 100 ppm (\pm 0, 0.092, 0.184 and 0.369 mg/L) Males: minimum 28 D Females: \pm 45 D Anonymous 37, 2003	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: $0.6 < C \leq 3$ mg/L/6 h/d Cat. 1: $C \leq 0.6$ mg/L/6 h/d For \pm 45 D of exposure Cat. 2: $0.4 < C \leq 2$ mg/L/6 h/d Cat. 1: $C \leq 0.4$ mg/L/6 h/d	Slight decrease MetHb in M Only very low doses tested
Nitromethane			
16-day repeated dose toxicity study Inhalation route Rat (F344) 5/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (\pm 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	Not examined	Cat. 2: $1.2 < C \leq 6$ mg/L/6 h/d Cat. 1: $C \leq 1.2$ 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 (\pm 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	Not examined	Cat. 2: $1.2 < C \leq 6$ mg/L/6 h/d Cat. 1: $C \leq 1.2$ mg/L/6 h/d	/
Nitroethane			
No subacute toxicity study available	/	/	/

Sub-chronic exposure:

Table 127: Summary data on hematological effects observed after a 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	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 \geq 375 ppm	Cat. 2: $0.2 < C \leq 1$ mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Effects observed at doses supporting Cat. 2

CLH REPORT FOR NITROMETHANE

0, 94, 188, 375, 750 and 1500 ppm (\pm 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	Platelets count mildly to markedly increased in all treated group MetHb increased in M at \geq 375 ppm and in F at 750 ppm and 1500 ppm		
13-week repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (\pm 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	Minimal extramedullary hematopoiesis in spleen at 1500 ppm (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) Hematological examination not performed	Cat. 2: $0.2 < C \leq 1$ mg/L/6 h/d; dose of 188 and 375 ppm relevant for classification Cat. 1: ≤ 0.2 mg/L/6 h/d	No effects observed at doses warranting a classification However, hematological examination not performed
Sub-chronic repeated dose toxicity study Inhalation route Rat (SD) 50 M/dose 100 and 750 ppm (\pm 0.25 and 1.875 mg/L) 13 w of exposure Lewis <i>et al.</i> , 1977	750 ppm: sign. Decrease in Ht and Hb MetHb not sign. modified	Cat. 2 for 13-week exposure: $0.2 < C \leq 1$ mg/L/6 h/d	No classification
Sub-chronic repeated dose toxicity study Inhalation route Rabbit (NZW) 15 M/dose 100 and 750 ppm (\pm 0.25 and 1.875 mg/L) 13 w of exposure Lewis <i>et al.</i> , 1977	Hb reduced at 1 month at the highest dose (no info for 100 ppm)	Cat. 2 for 13-week exposure: $0.2 < C \leq 1$ mg/L/6 h/d For the 1-month time point: $0.6 < C \leq 3$ 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 (\pm 0, 0.3, 1.0 and 3.0 mg/L) 92 D	1000 ppm: sign. increase in methemoglobin levels (associated with cyanosis), 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 \geq 100 ppm and in 5, 4 and 5 F, resp. at 100, 350 and 1000 ppm) + extramedullary hematopoiesis (in all M at \geq 100 ppm and in 1, 2 and 1 F, resp. at 100, 350 and 1000 ppm)	For interim kill: 30-day Cat. 2: $0.6 < C \leq 3$ mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill (90-day) Cat. 2: $0.2 < C \leq 1$ mg/L/6 h/d	Supporting Cat. 2 based on microscopic effects

CLH REPORT FOR NITROMETHANE

Anonymous 26, 1982		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 ($\pm 0, 0.3, 1.0$ and 3.0 mg/L) 93 D Anonymous 26, 1982	MetHb sign increase at 1000 ppm in F Heinz bodies sign. \uparrow in both sexes at 1000 ppm (tend to \uparrow at low and mid doses) No splenic microscopic effects observed	For interim kill: 30-day Cat. 2: $0.6 < C \leq 3$ mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill (90-day) Cat. 2: $0.2 < C \leq 1$ mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	No classification

Chronic exposure:

Table 128: 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 ($\pm 0, 0.235, 0.47$ and 0.94 mg/L) 2 y of exposure NTP, 1997	Hematological examination not performed	Cat. 2: $0.025 \leq C \leq 0.125$ 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 ($\pm 0, 0.47, 0.94$ and 1.87 mg/L) 2 y of exposure NTP, 1997	Hematological examination not performed	Cat. 2: $0.025 \leq C \leq 0.125$ 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)	No effects observed However MetHb not examined	Cat. 2: $0.15 < C \leq 0.75$ mg/L/6 h/d Cat. 1: $C \leq 0.15$ mg/L/6 h/d	/

Anonymous 35, 1986			
--------------------	--	--	--

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-nitropropane
2-NP	2-nitropropane
5 HIAA	5-hydroxyindolacetic acid
Abs	Absolute
ADME	Absorption, Distribution, Metabolism, and Excretion
ALP	Alkaline phosphatase
ALT	Alanine Transaminase
Alv	Alveolar
Approx.	Approximately
AST	Aspartate Transaminase
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 cells	Chinese Hamster Ovary cells
Chrom.	Chromosome
CMC	Carboxymethylcellulose
Conc.	Concentration
Corresp.	Corresponding
CP	Cyclophosphamide
CT	Clotting time
D or d	Day
DMSO	Dimethyl sulphoxide
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

CLH REPORT FOR NITROMETHANE

g	Gram
GD	Gestation Day
GLP	Good Laboratory Practices
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
LC0	Lethal concentration 0%
LC50	Lethal concentration 50%
LC100	Lethal concentration 100%
LCLo	Lethal Concentration Low
LD0	Lethal Dose 0%
LD50	Lethal Dose 50%
Liq.	Liquide
LOAEC	Low Adverse Effect Concentration
LOAEL	Low 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	Micronucleated binucleated cells
Multifoc.	Multifocal
N or Nb or No	Number
NA	Not applicable
NC	Negative control
NCE	Normochromatic Erythrocytes
ND	Not determined
NE	nitroethane
Neg	Negative

CLH REPORT FOR NITROMETHANE

NM	Nitromethane
NOAEC	No Adverse Effect Concentration
NOAEL	No Adverse Effect Level
NOEC	No Effect concentration
Nucl.	Nucleated
NZW	New Zealand White
O.E.	Olfactory epithelium
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 Cells
RCS	Relative cell survival
Rel	Relative
Repr.	Reprotoxicity
Resp.	Respectively
Resp. epith	Respiratory epithelium
RPE	Relative plating efficiency
S.	Slight
<i>S. Typh.</i>	Salmonella typhimurium
SCE	Sister Chromatid Exchange
SD	Sprague-Dawley
SDH	Serine dehydratase
SEM	Standard error of the Mean
SHE cells	Syrian Hamster Embryo cells
Sign.	Significant(-ly)
St. Dev.	Standard deviation
STOT RE	Specific target organ toxicity – repeated exposure
STOT SE	Specific target organ toxicity – single exposure
T3	Triiodothyronine
T4	Thyroxine
TCA	Tricarboxylic 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

Adelakun S.A., Ukwenya V.O., Ogunlade B.S., Aniah J.A. and Ibiayo A.G., 2019, Nitrite-induced testicular toxicity in rats: therapeutic potential of Walnut oil, in JBRA Assist Reprod, 23(1), 15-23.

Aly H., Mansour A., Abo-Saleem O., Abd-Ellah H. and Abdel-Naim A., 2010, Potential testicular toxicity of sodium nitrate in adult rats, in Food Chem Toxicol., 48(2), 572-8.

Bretherick L., 1990, Handbook of reactive chemical hazards, 4th Ed.

Bretherick L., 2007, Handbook of reactive chemical hazards, 7th Ed.

Budavari S., 1989, The Merck Index – Encyclopedia of Chemicals, drugs and Biologicals.

Chemiakaarten, 1997, 12th Ed.

Clausen H.S., Ebdrup N.H., Barsoe I.M., Lyngso J., Schullehner J., Ramlau-Hansen C.H., Bay B. and Knudsen U.B., 2020, Association between drinking water nitrate and adverse reproductive outcomes: a systematic PPRISMA review, in Water, 12(2287).

Daubert T.E. and Danner R.P., 1989, Physical and Thermodynamic Properties of pure chemicals data compilation.

Davenport J., Hagquist W. and Rankin G., 1970, The symmetrical maze, in Behaviourak Research methds and instrumentation, 2(3), 112.

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

De Groef B., Decallonne B.R., Van der Geyten S., Darras V.M. and Bouillon R., 2006, Perchlorate versus other environmental sodium/iodide symporter inhibitors: Potential thyroid-related health effects. Eur. J. Endocrinol. 155, 17–25.

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

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

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

Hebb D. and Williams K., 1946, *Journal of Genetic Psychology*, 59-65.

Fiala E.S., Conaway C.C., Biles W.T. and Johnsson B., 1987, Enhanced mutagenicity of 2-nitropropane nitronate with respect to 2-nitropropane: Possible involvement of free radical species, *Mutat. Res.* 179, 15-22.

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

Health Canada, Guidelines for Canadian Drinking Water Quality, Guideline Technical Document, Nitrate and Nitrite, 2013.

IPCS Inchem, 1997.

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

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

Lide D.R., 2000, *The CRC Handbook of Chemistry and Physics*, 81th Ed.

Lide D.R., 2003, *The CRC Handbook of Chemistry and Physics*, 84th Ed.

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

MacGregor J.T., Wehr C.M. and Langlois R.G., 1983, A simple fluorescent staining procedure for micronuclei and RA in erythrocytes using Hoescht 33258 and pyronin Y, *Mutat. Res.*, 120, 269-275.

Margolin B.H., Risko K.J., Frome E.L. and Tice R.R., 1990, A general purpose statistical analysis program for micronucleus assay data, App. 2: Micronucleus data management and analysis version 1.4a, Integrated Laboratory Systems, Research Triangle Park, NC.

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

National Fire protection Association, 2002, *Fire protection guide to hazardous materials*, 13th Ed.

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

Nobunaga T., Tokugawa Y., Hashimoto K., Kubota Y., Sawai K., Shimoya K., Takemura M., Matsuzaki N., Azuma C. and Saji F., 1996, Elevated nitric oxide concentration in the seminal plasma of infertile males: nitric oxide inhibits sperm motility, in *Am J Reprod Immunol.*, 36(4), 193-7.

Page *et al.*, 2001. Peripheral neuropathy in workers exposed to nitromethane, *American Journal of Industrial Medicine*, 40, 107-113.

Panesar N. and Chan K., 2000. Decreased Steroid Hormone Synthesis from Inorganic Nitrite and Nitrate: Studies *in vitro* and *in vivo*, in *Toxicology and Applied Pharmacology*, 169(3), 222-230.

Radikova Z., Tajtakova M., Kocan A., Trnovec T., Sebkova E., Klimes I. and Langer P. 2008. Possible effects of environmental nitrates and toxic organochlorines on human thyroid in highly polluted areas in Slovakia. *Thyroid Off. J. Am. Thyroid Assoc.*, 18, 353–362.

Revelli A., Bergandi L., Massobrio M., Lindblom B., Bosia A. and Ghigo D., 2001. The concentration of nitrite in seminal plasma does not correlate with sperm concentration, sperm motility, leukocytospermia, or sperm culture, in *Fertil Steril*, 76(3), 496-500.

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

Schmid W., 1976. The micronucleus test for cytogenic analysis, *Chemical Mutagens, Principles and Methods for their detection*, Ed. A. Hollaender, Plenum Press, NY, Vol 4, 31-53.

Smith D. and Anderson R., 2013. Toxicity and Metabolism of Nitroalkanes and Substituted Nitroalkanes. *Journal of Agric. Food Chem.*, 61, 763–779.

Tajtakova M., Semanova Z., Tomkova Z., Szokeova E., Majoros J., Radikova Z., Sebkova E., Klimes I. and Langer P., 2006. Increased thyroid volume and frequency of thyroid disorders signs in schoolchildren from nitrate polluted area. *Chemosphere*, 62, 559–564.

Toopa E.E., 1955, Physical properties of the Nitroparaffins Expansions of data reported in CRMR No.2055, density equations, viscosity and evaluation of literature data, *Chemical Research Memorandum Report No. 2082*.

Van Maanen J.M., Welle I.J., Hageman G., Dallinga J.W., Mertens P.L. and Kleinjans J.C., 1996. Nitrate contamination of drinking water: Relationship with HPRT variant frequency in lymphocyte DNA and urinary excretion of N-nitrosamines. *Environ. Health Perspect.*, 104, 522–528.

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

Webb *et al.*, 2002. Occupational Allergic contact dermatitis to nitromethane, Elsevier science USA.

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

WHO, 2016. Nitrate and Nitrite in Drinking-water, Draft background document for development of WHO Guidelines for Drinking-water Quality, 2016.

Zraly Z., Bendova J., Svecova D., Faldikova L., Veznik Z. and Zajicova A., 1997. Effects of oral intake of nitrates on reproductive functions of bulls, in *Vet Med*, 42(12), 345-54.