CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Nitroethane

EC Number: **201-188-9**

CAS Number: **79-24-3**

Index Number: **609-035-00-1**

Contact details for dossier submitter:

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

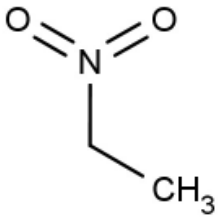
Version number: 4 **Date: December 2023**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Nitroethane
Other names (usual name, trade name, abbreviation)	1-nitroethane Ethane, nitro- Nitroethan
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	201-188-9
EC name (if available and appropriate)	Nitroethane
CAS number (if available)	79-24-3
Other identity code (if available)	/
Molecular formula	C2H5NO2
Structural formula	
SMILES notation (if available)	/
Molecular weight or molecular weight range	75.07 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)	≥ 99.9 % (w/w)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Nitroethane	/	Flam. Liq. 3, H226	Flam. Liq. 3, H226

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
(EC n° 201-188-9)		Acute Tox. 4*, H302 Acute Tox. 4*, H332	Acute Tox. 4, H302 Acute Tox. 4, H332

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

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

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 4: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	609-035-00-1	nitroethane	201-188-9	79-24-3	Flam. Liq. 3 Acute Tox. 4* Acute Tox. 4*	H226 H302 H332	GHS02 GHS07 Wng	H226 H302 H332			
Dossier submitters proposal	609-035-00-1	nitroethane	201-188-9	79-24-3	Retain: Flam. Liq. 3 Modify: Acute Tox. 4 Acute Tox. 4 Add: Carc. 1B Repr. 1B STOT RE 2	Retain: H226 H302 H332 Add: H350 H360Df H373 (blood, respiratory tract and nervous system)	Retain: GHS02 GHS07 Add: GHS08 Remove: Wng Modify: Dgr	Retain: H302 H332 H226 Add: H350 H360Df H373 (blood, respiratory tract and nervous system)		Add: ATE (oral) = 1080 mg/kg bw ATE (inhalation) = 18.50 mg/L	
Resulting Annex VI entry if agreed by RAC and COM	609-035-00-1	nitroethane	201-188-9	79-24-3	Flam. Liq. 3 Acute Tox. 4 Acute Tox. 4 Carc. 1B Repr. 1B STOT RE 2	H226 H302 H332 H350 H360Df H373 (blood, respiratory tract and nervous system)	GHS02 GHS07 GHS08 Dgr	H226 H302 H332 H350 H360Df H373 (blood, respiratory tract and nervous system)		ATE (oral) = 1080 mg/kg bw ATE (inhalation) = 18.50 mg/L	

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. 4, H332**	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

Nitroethane is a chemical substance which is registered under REACH (1907/2006/EC). The substance is listed in annex VI of CLP (609-035-00-1) with following classification:

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

Several self classifications are reported in the C&L inventory (consulted on the 30-11-2023) : 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
Repr. 2, H361 (inhalation)
Repr. 2, H361fd
STOT SE 3, H335 (respiratory system) (inhalation)
Eye Irrit. 2, H319
Aquatic Chronic 2, H411
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 (Repro. 2, H361). Furthermore, new data are available to assess the classification: 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.
* STOT RE: Disagreement by DS with current self-classification not including classification for STOT RE.

5 IDENTIFIED USES

Used in coatings.

6 DATA SOURCES

Registration dossier (last consultation by the DS: November 2022; https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/10513)

C&L inventory: last consulted by the DS: November 2023

Full study report

7 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101.3 kPa	Colourless organic liquid	Anonymous 18 (2011)	1 (reliable without restriction) GLP
Melting/freezing point	-89.52 °C at 1 atm	Anonymous 19 (1955)	2 (reliable with restriction) Non-GLP, non-guideline Cryoscopic method
	-89.5 °C	Lide D.R., CRC Handbook of Data on Organic Compounds Volume I, 3d ed., (1994)	2 (reliable with restriction) Data from peer reviewed handbook
Boiling point	114.07 °C at 760 mmHg	Anonymous 19 (1955)	2 (reliable with restriction) Non-GLP, non-guideline Ebulliometer
	114 °C	Lide D.R., CRC Handbook of Data on Organic Compounds Volume I, 3d ed., 1994	2 (reliable with restriction) Data from peer reviewed handbook
Relative density	1.051 at 20 °C 1.045 at 25 °C 1.039 at 30 °C	Anonymous 19 (1955)	2 (reliable with restriction) Non-GLP, non-guideline Pycnometer method
	1.045 at 25 °C	Lide D.R., CRC Handbook of Data on Organic Compounds Volume I, 3d ed., 1994	2 (reliable with restriction) Data from peer reviewed handbook
Vapour pressure	20.9 mmHg at 25 °C	Anonymous 19 (1955)	2 (reliable with restriction) Non-GLP, non-guideline Ebulliometer
	20.8 at 25 °C	Daubert, T.E. and R.P. Danner, Physical and thermodynamic Properties of Pure Chemicals Data Compilation (1989)	2 (reliable with restrictions) Non-guideline Data obtained from a peer reviewed handbook
Surface tension	72.0 mN/m at 21.4+/-0.5 °C	Anonymous 20 (2011)	1 (reliable without restriction) GLP EU A.5 (Surface tension) OECD harmonised ring method

Property	Value	Reference	Comment (e.g. measured or estimated)
Water solubility	48 g/L at 25 °C	Yalkowsky S.H. CRC Handbook of aqueous solubility data: an extensive compilation of aqueous solubility data for organic compounds, 2003	2 (reliable with restrictions) Peer reviewed data
Partition coefficient n-octanol/water	Log Kow = 1.45 at 22.4 °C and pH ca.7	Anonymous 20 (2011)	1 (reliable without restriction) GLP EU A.8 (partition coefficient) Shake flask method to: flask method
Flash point	31°C +/- 2 °C at 102.55 kPa	Anonymous 18 (2011)	1 (reliable without restriction) GLP EU A.9 (Falsh point) Equilibrium method closed cup
	28 °C at 760 mmHg	Fire Protection Guide to Hazardous Materials. 13 ed, 2002	2 (reliable with restrictions) Closed cup Data from handbook or collection of data with peer review
	28 °C at 760 mmHg	IPCS Inchem (1998)	2 (reliable with restrictions) Closed cup Data from handbook or collection of data with peer review
	28 °C at 760 mmHg	Chemiekaarten, 12 ed, 1997	2 (reliable with restrictions) Data from handbook or collection of data with peer review
Flammability	/	/	/
Explosive properties	Non-explosive	Anonymous 18 (2011)	1 (reliable without restriction) GLP EU A.14 (Explosive properties)
Self-ignition temperature	416 °C at 99.98-100.1 kPa	Anonymous 18 (2011)	1 (reliable without restriction) GLP EU A.15 (auto-ignition temperature (Liquid and Gases))
	414 °C at 760 mmHg	Fire Protection Guide to Hazardous Materials. 13 ed, (2002)	2 (reliable with restrictions) Data from handbook or collection of data with peer review
	414 °C at 760 mmHg	IPCS Inchem (1998)	2 (reliable with restrictions) Data from handbook or collection of data with peer review

Property	Value	Reference	Comment (e.g. measured or estimated)
	410 °C at 760 mmHg	Chemiekaarten, 12 ed (1997)	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. 4th ed., 1990	2 (reliable with restrictions) Read across Data from handbook or collection of data with peer review
Granulometry	Substance is a liquid	/	/
Stability in organic solvents and identity of relevant degradation products	/	/	/
Dissociation constant	pKa = Ca. 8.57	Anonymous 20 (2011)	2 (reliable with restrictions) QSAR (I-Lab 2.0)
Viscosity	0.64 mPa s at 25 °C (dynamic viscosity)	Anonymous 19 (1955)	2 (reliable with restriction) Non-GLP, non-guideline

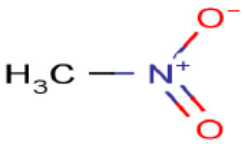
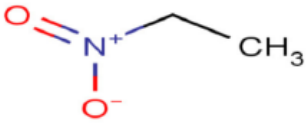
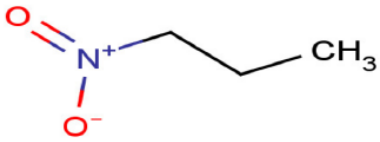
Read-across justification between nitromethane, nitroethane and 1-nitropropane:

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

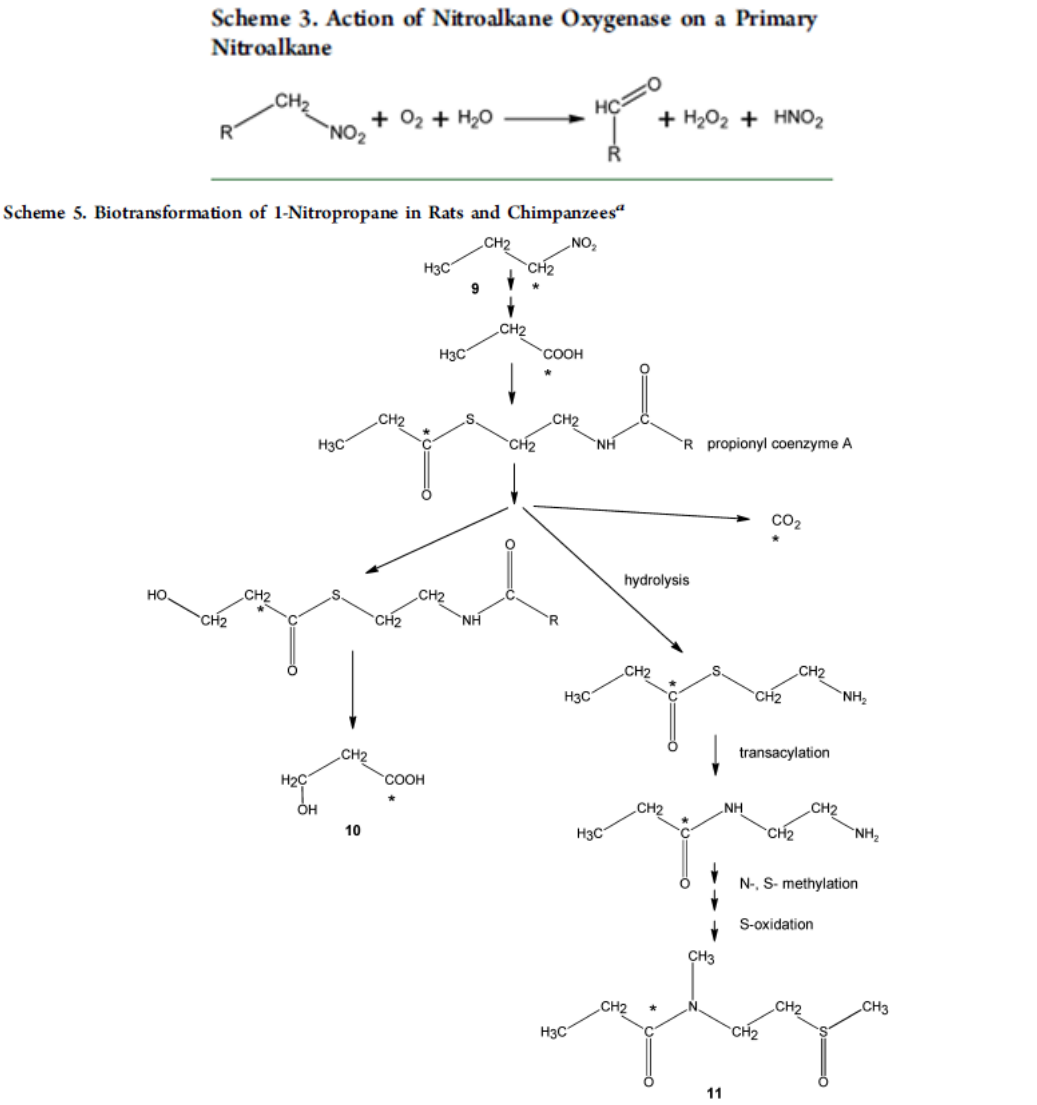


Figure 1: Biotransformation of 1-nitropropane as proposed by Smith & Anderson (2013)

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

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

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

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

radiolabeled fraction was 1-nitropropane and it represented around 10 % of the total radioactive dosed compound.

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

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

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

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

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

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

The classification proposal for carcinogenicity of nitroethane and nitropropane is fully based on read-across from nitromethane because the available studies on nitroethane and nitropropane are uninformative due to too low dosing and too low animal number. Thus, the key studies for the assessment of carcinogenicity are the 2-year studies in mice and rats on nitromethane for all category members and Carc. 1B; H350 are proposed for nitromethane, nitroethane and nitropropane in individual dossiers.

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

The classification proposal for developmental toxicity of nitroethane and nitropropane is fully based on read-across from nitromethane OECD TG 414 study in rats, because there is no prenatal developmental toxicity

study available on nitroethane and 1-nitropropane. Overall, the available data on nitromethane is considered to support Repr. 1B; H360D for nitromethane, nitroethane and nitropropane and these classifications are proposed in individual dossiers.

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

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this CLH dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
The Metabolism of Nitroparaffins: II The Metabolic Products of Nitroethane (In vitro and in vivo metabolism in rabbit blood) No guideline Not GLP-compliant Rabbit: strain not specified Sex: not specified 1/sex/dose Intravenous Single dose: 1000 mg	Metabolites: Acetaldehyde and Nitrite was found in blood following intravenous administration	/	Scott E. W., 1942
The Metabolism of Mononitroparaffins: III The Concentration of Nitroethane, Nitrite and Nitrate in the Blood of Rabbits during Exposure by Inhalation and Oral Administration No guideline Not GLP-compliant Rabbit: strain not specified Sex: male	The concentration of nitrate and nitrite increased gradually in the blood of rabbits during exposure to nitroethane by inhalation or oral administration	/	Scott E.W., 1943

Method	Results	Remarks	Reference
1/dose Inhalation or oral Inhalation: 1.24-1.47, 0.29, 0.27 and 1.19 % of inhaled air for 6, 5, 9 and (unknown) hours of exposure, respectively. Oral: single dose of 3.15 g Vehicle: not specified			
Skin absorption and metabolism Toxicokinetics study of 14C-nitroethane No guideline GLP 2 Female Rhesus Monkeys (1/dose) Doses: 15.46 mg and 11.85 mg (not enough material to expose both animals to the same dose) Reliability 2 (according to the registration dossier)	Absorption through skin occurred only in negligible amounts	/	Anonymous 21, 1990

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

In a non-guideline study (Scott, 1942), nitroethane metabolism was assessed *in vitro* (with and without addition of H_2O_2) and *in vivo*. Oxidation of nitroethane was evaluated *in vitro* with H_2O_2. 25 ml of citrated blood, with addition of 210 mg nitroethane diluted in 100 ml water, was finaly mixed with 6 ml of 5 % H_2O_2. Protein precipitation was then achieved with tungstic acid. The same design was applied on a blood sample without H_2O_2, and one blood sample without H_2O_2 nor nitroethane. Samples stayed 3 h at rest before it was diluted.

- with H_2O_2: the solution contained 77 % of nitroethane and acetaldehyde equivalent to 16 % of the nitroethane, causing a deficience of 7 % in the recovery.

- without H_2O_2: 80 % nitroethane and acetaldehyde equivalent to 18.5 % of the nitroethane. No acetaldehyde was detected in a control blood sample (no added nitroethane).

One rabbit was also given intravenously 1000 mg nitroethane and 3 blood samples were taken after at various time points: 30, 120, and 300 minutes. 58.5 and 68.0 mg nitroethane and 0.6 and 0.62 mg nitrite per 100 mL blood were observed after 30 and 120 minutes, respectively. After 5 hours, nearly 0 mg/100 mL was detected, for both substances, although the nitrite color of this sample was more intense than the control's. Given the exposure level to nitroethane and the results previously obtained for acetaldehyde, the percentage of nitrite was relatively low. The authors interpreted that finding with the readiness of the reaction oxyhemoglobin-nitrite, from which nitrate appears.

A sub-study was designed in order to test this hypothesis. A rabbit was exposed to 50 mg $NaNO_2$ by intravenous injection. Resulting nitrite level was 3.88, 1.96, 0.60 and 0.28 mg/100 mL blood after 5, 45, 120 and 240 minutes, respectively. Results show that nitrite ion in blood is first rapidly removed until a certain point is reached, after that, the reduction in concentration becomes more gradual.

In a second experiment (Scott, 1943), one rabbit (2.5 kg) was exposed to 3.15 g nitroethane via oral administration and 4 rabbits anesthetized (sodium barbital) were exposed by inhalation (see Table 9 below).

Table 9: Rabbits BW and inhalation exposure parameters

N	Body weight (kg)	Nitroethane (%) in air	Duration (h)
1	2.9	1.24 – 1.47	6
2	3.6	0.29	5
3	2.75	0.27	9
4	2.5	1.19	Not specified

Nitrate levels in rabbit blood increased gradually during exposure (inhalation or oral) to nitroethane.

Table 10: Blood concentrations of nitrate and nitroethane after oral administration of nitroethane

Time from exposure (min)	Nitrate (mg/100 mL)	Nitroethane (mg/100 mL)
25	4.0	115
63	4.0	107
122	5.0	92
186	11.0	105
243	32.0	120
366	20.0	72

Table 11: Blood concentrations of nitrate and nitroethane after inhalation of nitroethane

No	Exposure	Rabbit BW (kg)	Exposure to Nitroethane (%)	Nitrate (mg/100 mL)	Nitroethane (mg/100 mL)
1	Inhalation	2.9	1.24 - 1.47	21	270
2		3.6	0.29	8.4 and 16 after 300 and 380 min, resp.	21
3		2.75	0.27	18	36
4		2.5	1.19	0.60	

In rabbit No. 3, 19 and 10 mg nitrate/dL were found in two urine samples.

Each rabbit was also administrated by intravenous injection of other nitroparaffins (1 millimole, in aqueous solution) and nitrite levels in blood were determined at regular intervals.

Table 12: Blood Levels of Nitrate following administration of various Nitroparaffins.

	Nitrate/100 mL blood (mg)				
Compound Used	After 5 min	After 30 min	After 1 h	After 2 h	After 3 h
Nitromethane	Trace	N.D.	Trace	Trace	N.D.
Nitroethane	0.12	N.D.	0.26	0.24	0.33
1 -Nitropropane	0.07	N.D.	0.17	0.16	0.12
2 -Nitropropane	0.33	N.D.	0.10	0.23	N.D.
1 -Nitrobutane	N.D.	0.040	0.014	0.004	N.D.
2 -Nitrobutane	N.D.	0.047	0.042	0.034	N.D.
2 -Nitro-2-methylpropane	N.D.	Nil	Nil	Nil	N.D.
Sodium Nitrite (69 mg)	3.65	N.D.	1.20	N.D.	N.D.

N.D.: not determined

Based on all these results, the bioaccumulative potential of nitroethane could not be assessed.

In a skin absorption and metabolism study with 14C-nitroethane (Anonymous 21, 1990), 2 female Rhesus monkeys were exposed to either 300 µL (15.46 mg) or 230 µL (11.85 mg) nitroethane diluted in ethanol or an ethanol/ether solution. 72 h before exposure, a zone in the back of each monkey was shaved. A 20 cm^2 zone was marked with tattoo ink to specify the test zone. 24 h prior to exposure, test site was cleaned with isopropanol. Animals were sedated (ketamine HCl) and a catheter was inserted in the leg vein. Nitroethane was evenly applied on the test site and an occlusive bandage was taped over the zone. 12 h after exposure, monkeys were sedated once again, the bandage was removed and the test site was cleaned (3x with soap and water, one last time with acetone). Finally, 72 h after exposure, animals were sedated and the test zone + adjacent 1 cm were excised. Subcutaneous fat was removed from the skin and both were seighed separately. Urine,feces and blood samples were collected at several time points and analysed at least in triplicates.

- Urine: 0-2, 2-4, 4-6, 6-8, 8-10 and 10-12 and at 12-hour intervals thereafter
- Feces: 0-4, 4-8, 8-12 hours and at 12-hour intervals thereafter
- Blood: 0.33, 0.66, 1, 2, 3, 4, 6, 8, 10 and 12 hours and at 12-hour intervals thereafter

Subcutaneous fat, skin and swabs extracts were also analysed.

Results showed that absorption through the skin occurred at a negligible level. No sign of toxicity was observed in either monkey. Average excretion was 16.2 µg (77.2 % of which was found in urine). After 48 h, 91.4 % of total urine radioactivity was excreted. 22.8 % of total excreted radioactivity was recovered in the feces, within 48h. In blood, average maximal level of 41.3 ng nitroethane/ml blood. Nitroethane was not detected in blood after 24 h. In the skin, 4.05 µg of nitroethane was recovered (0.029 %) and a much lower dose in the fat (0.001 %). The high loss of the test material (99.79 %) was estimated to be due to the very high volatility of nitroethane and evaporation from the test zone. Plus, exhaled radioactivity was not trapped in the present test conditions.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Table 13: 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
Acute oral toxicity study Equivalent to OECD TG 401 GLP Oral gavage in solution of 1 % carboxymethyl cellulose 14 d observation period Reliability 1 (according to the registration dossier)	Rat (Cox-SD albino white) 10/sex in gp I 10 females in gp II, III, IV ≥ 6-wk old BW: 204 ± 17 g in average	**Nitroethane** Purity: 96.52 % Impurities: 0.012 % Nitromethane 3.38 % Nitropropane	Single exposure 0, 560, 800, 1100, 1600 and 2300 mg/kg bw (Gp I) Additional groups of females: Gp II (950 mg/kg bw) Gp III (1000 and 1050 mg/kg bw) Gp IV (950, 1050 and 1250 mg/kg bw)	**LD50 (males): 1428 mg/kg bw** **LD50 (females): 1083 mg/kg bw**	Anonymous 22, 1982

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 No guideline Not GLP Oral gavage 14 d observation period Reliability 2 (according to the registration dossier, however poorly reported data)	Rat (strain not specified) Male only 2-3 rats/dose	**Nitroethane** Purity unknown	126, 252, 500, 1000 and 2000 mg/kg bw	**LD50 (male)** **1000 mg/kg bw**	Anonymous 23, 1964
Physiological response No guideline Not GLP Oral gavage Deficient reporting Reliability 2 (according to the registration dossier, however poor quality of the full study report pdf file)	Rabbit (strain not specified) Sex not specified	**Nitroethane** Purity unknown	Not stated At least 500 and 750 mg/kg bw	**500 < LD50 < 750 mg/kg bw**	Machle *et al.*, 1940
Disregarded study Insufficient reporting for assessment	Rat (strain not specified) Sex not specified	Nitroethane Purity unknown	At least 1000 and 2000 mg/kg bw	**LD0: 1000 mg/kg bw** **LD50: 1625 ± 193 mg/kg bw** **LD100: 2000 mg/kg bw**	Anonymous 24, 1960
Disregarded study Insufficient reporting for assessment	Rat Strain not specified Sex not specified	Nitroethane Purity unknown	280 and 420 mg/kg bw	**LD50: 420 mg/kg bw**	Anonymous 25, 1956

No human data or other relevant studies available.

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

In a study equivalent to an oral acute toxicity test (Anonymous 22, 1982), male and female rats received either 0, 560, 800, 1100, 1600 or 2300 mg/kg bw of nitroethane in 1 % carboxymethyl cellulose solution by oral gavage (Gp I). 3 others groups of only 10 female rats were exposed to the same test substance at levels of either 950 mg/kg bw (Gp II), 1000 or 1050 mg/kg bw (Gp III) or 950, 1050 or 1250 mg/kg bw (Gp IV). Animals were then observed for up to 14 days. LD50 were determined as 1428 mg/kg bw in males (IC95: 1232 – 1657 mg/kg bw) and 1083 mg/kg bw in females (IC95: 991-1167 mg/kg bw). Lethargy and ataxia were

reported in both sexes when animals were exposed to more than 800 and 1000 mg/kg bw, respectively. Those clinical signs appeared within 4 hours after exposure and lasted 2-3 days. Furthermore, anorexia and bloody nostrils were reported at day 1 in females, as well as blood in feces by day 2 and 3. By day 7, remaining animals returned to their normal behaviour. At necropsy, several intestinal haemorrhages were observed in animals dead within 14 d after exposure, while lung infections were detected in some surviving animals (including controls) after the observation period.

Table 14: Mortality rate

Doses (mg/kg bw)		0	560	800	1100	1600	2300
Gp I ♂	Mortality	0/10	0/10	0/10	0/10	7/10	10/10
	BWG (g)	50	40	42	43	43	/
	Lung infection	0/1	2/10	1/10	0/10	1/10	/
Gp I ♀	Mortality	0/10	0/10	0/10	8/10	10/10	10/10
	Number/D observation				1/1; 7/2	7/1; 3/2	4/1; 5/2; 1/3
	BWG (g)	23	13	16	18	/	/
	Lung infection	0/10	0/10	0/10	0/10	/	/
Doses	(mg/kg bw)	0	950	1000	1050	1250	/
Gp II ♀	Mortality	0/10	0/10	/	/	/	/
	BWG (g)	7	5	/	/	/	/
	Lung infection	1/10	0/10	/	/	/	/
Gp III ♀	Mortality	0/10	/	4/10	6/10	/	/
	Number/Day observation	/	/	/	1/1; 3/2; 1/3; 1/5	/	/
	BWG (g)	15	/	14	8	/	/
	Lung infection	1/10	/	0/10	0/10	/	/
Gp IV ♀	Mortality	0/10	0/10	/	0/10	7/10	/
	Number/Day observation	/	/	/	/	3/1; 1/2; 3/3	/
	BWG (g)	23	21	/	16	15	/
	Lung infection	5/10	4/10	/	1/10	0/10	/

In an oral acute toxicity study (Anonymous 23, 1964), nitroethane was admistered by oral gavage to male rats at dose of 126, 252, 500, 1000 and 2000 mg/kg bw. Mortality was noted in the 2 highest dose groups. LD50 was determined as 1000 mg/kg bw. At necropsy, kidney and liver examination revealed changes (see Table 15).

Table 15: Mortality rate, clinical and necropsy observations

Dose level (in mg/kg bw)	500	1000	2000
Mortality	0/2	1/2	3/3

Time of death, clinical signs and necropsy	Slight kidney and liver lesions observed during necropsy	Death occurred 6 d after exposure. Moderate liver and slight kidney lesions seen during necropsy	Drowsiness and prostration seen after exposure. 2 animals died during the night and a third on the next day

In an oral acute toxicity study (Machle *et al.*, 1940), nitroethane was orally administered undiluted by gavage to rats at doses of 500 and 750 mg/kg bw. The body weight was daily followed until weightloss was regained then they were observed twice a week, then weekly. LC50 was determined to be between 500 and 750 mg/kg bw. 20 to 40 minutes after exposure, increasing weakness and collapse, unsteadiness, incoordination resulting in total ataxia as well as changes in respiration were noted. No significant changes in blood chemistry or color were reported.

Two studies are disregarded considering the unsufficience of data to conclude on the results given (Anonymous 24, 1960 and Anonymous 25, 1956).

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	All of the available studies conclude on a LD50 within the classification range for A**cute Toxicity Category 4** (300 ≤ ATE ≤ 2000 mg/kg bw). In that perspective, according to table "3.1.2.1. Classification criteria" of the Guidance on the Application of the CLP criteria, a classification as acute Tox. Category 4 is warranted. An **ATE of 1080 mg/kg bw**, derived from Anonymous 22 (1982), 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.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

The substance is currently classified as **Acute Tox. 4*, H302**. Considering the available data, DS proposes to modify the current classification as follow: **Acute Tox. 4, H302** (Harmful if swallowed). Based on CLP regulation, an **ATE of 1080 mg/kg bw** is warranted.

10.2 Acute toxicity - dermal route

Hazard class not evaluated in this CLH dossier

10.3 Acute toxicity - inhalation route

Table 16: 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
Acute inhalation toxicity study No guideline Not GLP Reliability 2 (according to the registration dossier)	Rat (Wistar) Sex not specified 8-10/dose group 250 g bw in average	**Nitroethane** Purity unknown Vapours	200, 550, 2200 and 13000 ppm corresp. to 0.625, 1.55, 6.8 and 40.6 mg/L, resp. Duration of exposure: 6 h (for 12 exposure) at 200 and 500 ppm, 6 h (for 5 exposure) at 2200 ppm and 6-7 h (for 1 exposure) at 13000 ppm	**6.8 < LC50 < 40.6 mg/L** **Calculated LC50(4h): 6025 ppm (approx. 18.50 mg/L)**	Dequidt *et al.*, 1973
Acute inhalation toxicity study No guideline No GLP Up to 3 weeks observation period Reliability 2 (according to the registration dossier)	Rat (strain not specified) Sex not specified 4/dose group	**Nitroethane** Purity unknown Vapours	Saturated atmosphere 0.2, 0.5 or 1 h	**LC100: saturated atmosphere for 1 h** **LC0: saturated atmosphere for 0.2 h**	Anonymous 23, 1964
Acute inhalation toxicity study No guideline No GLP Min. 2 months of observation after exposure Reliability 2 (according to the registration dossier, however poor quality of the full study report pdf file)	Rabbit (strain not specified) Sex not specified 2/dose group	**Nitroethane** Purity unknown	0, 500, 1000, 2500, 5000, 10000, 25000 and 30000 ppm equivalent to 0, 1.53, 3.07, 7.675, 15.35, 30.70, 76.75 and 92.11mg/L, resp. Duration of exposure: between 0.5 and 140 h	**LC100 (1.25h): 30000ppm,** **LC100(2h): 25000 ppm** **LC100(3h): 10000 ppm** **LC50(12h): 1000 ppm** **LC0(3h): 2500 ppm** **LC0(6h): 1000 ppm**	Machle *et al.*, 1940
Acute inhalation toxicity study No guideline Not GLP At least 6 months	Guinea pig (strain not specified) Sex not specified	**Nitroethane** Purity unknown	0, 500, 1000, 2500, 5000, 10000, 25000 and 30000 ppm equivalent to 0, 1.53, 3.07, 7.675,	**LC100 (1.25h): 30 000 ppm** **LC50 (3h): 10 000 ppm** **LC0: 25000,**	Machle *et al.*, 1940

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
of observation after exposure Reliability 2 (according to the registration dossier, however poor quality of the full study report pdf file)			15.35, 30.70, 76.75 and 92.11 mg/L, resp. Duration of exposure: between 0.5 and 140 h	**2500 and 1000 ppm after 2, 3 and 12 h, resp.**	
4-day inhalation toxicity study No guideline Not GLP 4 consecutive days of exposition Not mentioned in the registration dossier	Fischer 344 rats Male/female 5/sex/dose At least 8-wk old	**Nitroethane** Purity: 97.9 2 % Impurities: 0.01 % NM and 2.07 % 2-NP	0, 350, 1000, 2000 and 4000 ppm (corresp. to 0, 1.0, 3.0, 6.0 and 12.0 mg/L, resp.) Duration of exposure: 6 h/d for 4 d	The LC50 could not be determined as more than one exposition was performed	Anonymous 26, 1982
4-day inhalation toxicity study No guideline Not GLP 4 consecutive days of exposition Not mentioned in the registration dossier	B6C3F1 mice Male/female 5/sex/dose At least 6-wk old	**Nitroethane** Purity: 97.92 % Impurities: 0.01 % NM and 2.07 % 2-NP	0, 350, 1000, 2000 and 4000 ppm (corresp. to 0, 1.0, 3.0, 6.0 and 12.0 mg/L, resp.) Duration of exposure: 6 h/d for 4 d	The LC50 could not be determined as more than one exposition was performed	Anonymous 26, 1982
Disregarded study Insufficient data to interpret the results Reliability 4 (according to the registration dossier)	Rat (strain and sex: not specified) 10	Nitroethane Purity: not specified	2.25 mg/L 1 h	LC0: 2.25 mg/L	Anonymous 25, 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 inhalation acute toxicity study (Dequidt *et al.*, 1973), rats were exposed to nitroethane at different concentrations, for different periods of time (See Table 17). After all exposures were performed, methemoglobinemia and NO2 levels in predetermined tissues (liver, lung, heart, kidney) were assessed. All animals exposed to 13000 ppm died within the 6-7h of exposure. The LC100 was therefore set at 13000 ppm.

All animals survived when exposed to 100, 550 or 2200 ppm, even after multiple exposure sessions. Methemoglobin and NO2 levels are shown in Table 20, below. The LC50 was normalized for a 4 hour exposure and was calculated by the registrant at 6025 ppm, this is approximatively equivalent to 18.50 mg/L of nitroethane.

Table 17: Exposure levels, duration and mortality rate

Exposure level (ppm)	200	550	2200	13000
N of exposures	12	12	5	1
Duration/exposure (h)	6	6	6	6-7
Mortality rate (%)	0	0	0	100

Table 18: Methemoglobinemia and NO2 levels in tissues

Exposure level ppm)		200	550	2200	13000
N of exposures		12	12	5	1
MetHb (%)		0	0	0	2.84
NO2 content (µg/100 g tissue)	Liver	Trace	Trace	121	700
	Lung	Trace	60	14	192
	Heart	Trace	236	171	930
	kidney	Trace	Trace	55	255

In an inhalation acute toxicity study (Anonymous 23, 1964), rats were exposed to nitroethane in saturated atmosphere chambers for 0.2, 0.5 or 1 h. All animals exposed for 1h died, 75 % of animals exposed for 30 min died but all rats exposed for 12 min survived. All rats lost consciousness after being exposed for 1 h and died the next day. Drowsiness was observed on all animals exposed for 0.5 h, 2/4 rats died overnight and another rat died within the 3 weeks of observation. Severe liver lesions were seen on rats exposed for 0.5 h.

In Machle *et al.* study (1940), rabbits were exposed to nitroethane for varying durations, at different doses (see Table 19 below). It is not specified if the exposure was continuous or fractioned during several days. Surviving animals were followed up for minimum 2 months after exposure. All animals died after being exposed to 30000 ppm, 25000 ppm and 10000 ppm for 1.25, 2 and 3 hours, respectively. LC50 was determined to be 1000 ppm after 12 h of exposure. All animals survived after being exposed to 2500 ppm and 1000 ppm for 3 and 6 hours, respectively. Restlessness, uncomfortability, olfactory tract irritation, redness of lids, slight salivation, twitching and jerking moves regularly seen at high concentrations. Visceral and cerebral congestion were reported in all exposed rabbits, and to a lesser extent in control animals. Lung edema was noted in all animals which died at high concentrations, and upper olfactory tract irritation was diagnosed by local congestion. Edema, pallor or cloudy swelling as it is regularly seen after lethal doses were reported in kidneys and myocardium, and some unspecified other organs.

Table 19: Exposure parameters

Exposure level (ppm)	500	500	1000	1000	2500	5000	5000	10000	10000	25000	30000	30000	30000
Duration (h)	30	140	6	12	3	2	3	1	3	1	0.5	1	1.25

It is not specified if the exposure was continuous or fractioned during several days.

In Machle *et al.* (1940), guinea pigs were also exposed to nitroethane by inhalation for varying durations, at different doses (see Table 20) and animals were then observed for minimum 6 months. All animals exposed to 30000 ppm died after 1.25 h of exposure, 50 % of guinea pigs exposed to 10000 ppm died after 3 h and no death was recorded in groups exposed to 25000 ppm, 2500 ppm or 1000 ppm for 2, 3 or 2 h, respectively. Restlessness, uncomfortability, olfactory tract irritation, redness of lids, slight salivation, twitching and jerking moves regularly seen at high concentrations. Visceral and cerebral congestion were reported in all exposed guinea pigs, and to a lesser extent in control animals. Lung edema was noted in all animals which died at high concentrations, and upper olfactory tract irritation was diagnosed by local congestion. Edema, pallor or cloudy swelling as it is regularly seen after lethal doses were reported in kidneys and myocardium, and some unspecified other organs.

Table 20: Exposure parameters

Exposure level (ppm)	500	1000	1000	2500	5000	5000	10000	10000	25000	30000	30000	30000
Duration (h)	140	6	12	3	2	3	1	3	2	0.5	1	1.25

It is not specified if the exposure was continuous or fractioned during several days.

In an inhalation acute toxicity study (Anonymous 26, 1982), rats and mice were exposed to 0, 350, 1000, 2000 and 4000 ppm (0, 1.0, 3.0, 6.0 and 12.0 mg/L) of nitroethane for 6 h/d for 4 consecutive exposure days. As more than one exposition is performed, this study is used as a weight of evidence.
In rats, a significant decrease of body weight in the 1000, 2000 and 4000 ppm groups in both sexes was observed. After the first exposition, the males and females from the 2000 ppm groups showed drowsiness and all rats of the 4000 ppm groups died after two exposures. Before their deaths they revealed symptoms of anaesthesia, poor coordination, slow laboured respiration and dull dark-eyes with some exudate around them.
In mice, no significant body weight changes were detected. At 2000 ppm, the mice had a slightly laboured respiration (after the first exposition only), were drowsy and slightly uncoordinated. In this group, two deaths occurred after 3 exposures (one of each sex). In the 4000 ppm group, all mice showed slow laboured respiration and were anesthetized. None of them recovered before the 2 expositions and all of them died prior the 3rd exposure.

One study is disregarded due to insufficient reporting to conclude on the results given (Anonymous 26, 1956).

10.3.2 Comparison with the CLP criteria

According to the CLP criteria, a substance should be classified as Acute Tox. Category 4 when the ATE is estimated to be between 10.0 ≤ ATE ≤ 20.0 mg/L or 2500 ≤ ATE ≤ 20000 ppm. The mentioned LC50 by Machle *et al.* (1940) (1000 ppm for 12 h and 10 000 ppm for 3 h) and Dequidt *et al.* (1973) (6.8 < LC50 < 40.6 mg/L, calculated at 18.50 mg/L nitroethane) are in line with those criteria.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

The substance is currently classified as **Acute Tox. 4*, H332**. Considering the available data, DS proposes to modify the current classification as follow: **Acute Tox. 4, H332** (Harmful if inhaled). The chosen **ATE is 18.50 mg/L**, according to the study of Dequidt *et al.* (1973).

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 21: 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
NITROETHANE				
***In vitro* gene mutation test in bacteria** OECD TG 471 Non-GLP Reliability 2 (according to the registration dossier)	**Nitroethane**	*S. typh.* Deviations: only 4 out of 5 strains used (TA98, TA100, TA1535, TA1537) Test conc.: 100, 333.3, 1000, 3333.3 and 10000 µg/plate ± S9 Vehicle: DMSO No negative control Positive controls: -S9: 4-nitro-o-phenylenediamine for TA98, sodium azide for TA100 and TA1535 and 9-aminoacridine for TA1537 +S9: 2-aminoanthracene	Cytotoxicity observed in all 4 strains at 10000 µg/plate Precipitation was observed in the highest concentration tested in most experiments in all the strains Positive control: induced a clear increase in the number of revertants No significant increase in the frequency of revertant colonies was observed for any of the bacterial strains with any dose (up to 10 mg/plate), ± S9 **Negative**	Mortelmans *et al.*, 1986
***In vitro* gene mutation test in bacteria** Prior to OECD TG 471 GLP compliant Reliability 2 (according to registration dossier, however DS not access to raw data)	**Nitroethane**	*S. typh* 5 strains (TA98, TA100, TA1535, TA1537 and TA1538) Conc.: 55450 ppm (vapours) and at least 27725 ppm No vehicle Negative control: unspecified Positive control: -S9: 2-nitrofluorene for TA98 and TA1538; N-methyl-N'-nitro-N-nitrosoguanidine for TA100 and TA1535 and quinacrine mustard-2HCl for TA1537 +S9: 2-acetylaminofluorene for TA98 and TA1538; 2-anthramine for TA100 and TA1535 and 8-aminoquinoline for	Cytotoxicity was observed at 55450 ppm in TA1535 and TA1537. Therefore, a concentration of 27725 ppm was tested. No significant increase in the frequency of revertant colonies **Negative**	Anonymous 29, 1980

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		TA1537		
***In vitro* gene mutation study in mammalian cells** OECD TG 476 GLP-compliant Reliability 1 (according to 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 concentration tested Nitroethane was non-mutagenic both in absence and in presence of S9 metabolic fraction in the mammalian gene mutation test at concentrations up to the limit concentration. **Negative**	Anonymous 30, 2012
I*n vitro* gene mutation study in bacteria OECD TG 471 Non-GLP Reliability 2 (according to registration dossier, however reporting deficiencies)	**Nitroethane**	*S. typh.* Only 3 strains tested (TA98, TA100 and TA102) Conc.: not clearly specified but not up to 200 µmol/plate since nitromethane was toxic to bacteria at a 500 µmol/plate concentration Vehicle: DMSO + phosphate buffer (0.2 M, pH 7.4) Without met. act.	Negative without met. act. No cytotoxicity observed at any concentration. **Negative**	Dayal *et al.*, 1989
NITROMETHANE				
***In vitro* 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 *S. typh*. strains (TA98, TA100, TA1535 and TA1537) Test conc.: 100, 333.3, 1000, 3333.3 and 10000 µg/plate. +- S9 Vehicle: DMSO	No significant increase in the frequency of revertant colonies up to 10 mg/plate, +- S9 Only in TA100, cytotoxicity was observed at the highest concentration tested. **Negative**	Mortelmans *et al.*, 1986
***In vitro* gene mutation test in**	**Nitromethane**	Strain: 5 *S. typh.* strains (TA98, TA100, TA1535, TA1537 and TA1538)	No significant increase in the frequency of revertant colonies at	Anonymous

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
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)	No data on purity	Test conc.: A concentration resulting in saturated vapour atmosphere (47465 ppm) caused cytotoxicity in strains TA1535 and TA1537. For this reason, a concentration of 23732 ppm (118.7 mg/L) was tested. +- S9	23732 ppm, +- S9 **Negative**	27, 1980
***In vitro* chromosome aberration study in mammalian cells** CHO cells OECD TG 473 Non-GLP Reliability 2 (according to the registration dossier)	**Nitromethane** Purity unknown	Cell type: CHO cells Test conc.: No cytotoxicity was observed at limit concentration ➢ 11.5-hour treatment without S9: 1077, 2316 and 4980 µg/mL ➢ 2-hour treatment with S9 followed by 11.5 hours incubation with fresh medium: 1077, 2316 and 4980 µg/mL +- S9 Vehicle: distilled water	Negative +- S9 at concentrations as high as the limit concentration of 4980 µg/mL **Negative**	NTP, 1997
***In vitro* SCE assay in mammalian cells** CHO cells OECD TG 479 non-GLP Reliability 2 (according to the registration dossier)	**Nitromethane** Purity unknown	Cell type: CHO cells Test concentrations: No cytotoxicity was observed at limit concentration ➢ 26-hour treatment without S9: 497, 1655 and 4965 µg/mL then a 2-hour incubation without nitromethane ➢ 2-hour treatment with S9 then incubation was prolonged by 26 h: 497, 1655 and 4965 µg/mL +- S9 Vehicle: distilled water	No induction of SCE in CHO cells +- S9 at concentrations as high as the limit concentration of 4965 µg/mL **Negative**	NTP, 1997

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
***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 *S. typh.* strains (TA1535, TA1537 and TA1538) and 1 *Saccharomyces cerevisiae* (D4)	Disregarded study due to poor data reporting + test material not soluble under the treatment conditions	Anonymous 28, 1975
***In vitro* gene mutation test in bacteria** OECD TG 471 Deviation: only 3 strains tested without met. act. Non-GLP reliability 2 (according to the registration dossier, however reporting deficiencies)	**Nitromethane** Purity unknown	Strain: 3 *S.* typh. strains (TA98, TA100 and TA102) Test concentrations: Not specified but up to 200 µmol/plate. Only without S9 Vehicle: not specified	**Negative** without S9	Dayal *et al.*, 1989
***In vitro* cell transformation study in mammalian cells** EU Method B.21 Non-GLP Reliability 2 (according to the registration dossier)	**Nitromethane** Purity unknown	cells type: Syrian hamster embryo (SHE) Test conc.: 2000, 2500, 3000, 3500, 4000 and 5000 µg/mL (= top dose) ➢ Exposure for 24 h followed by 6-7 d of growth ➢ Exposure for 7 d Vehicle: DMSO	A dose-dependent significant increase in the morphological transformation frequency seen at the two highest concentrations tested. **Positive**	Kerckaert *et al.*, 1996
***In vitro* micronucleus test in SHE cells** Non-GLP	**Nitromethane** Purity unknown	Cells type: SHE cells Met. act.: not used Test concentrations:	Nitromethane did not induce an increased frequency of micronuclei in SHE cells. **Negative**	Gibson *et al.*, 1997

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Reliability 2 (according to the registration dossier)		- *With DMSO:* 0, 5.0, 5.5 and 6.0 µg/ml - *With Media:* 0, 3500, 4000, 5000 (µg/ml) Vehicle: DMSO or media		
		Results of an additional *in vitro* supporting study were provided but as the relevance of the study (i.e. induction of DNA damage and/or repair by measuring p53 levels in NCTC 929 cells with ELISA and Western blot analysis) is considered to be limited and results were negative, the study was not included in this report.		
1-NITROPROPANE				
***In vitro* gene mutation test in bacteria** With and without met. act. OECD TG 471 GLP Protocol adapted to volatile compound Reliability 1 (according to the registration dossier)	**1-nitropropane** Purity: 99 % Vehicle: DMSO	*S. typh.* TA98, TA100, TA1535 and TA1537 and *E. Coli* WP2uvrA- Test conc.: 20, 150, 500, 1500 and 5000 µg/plate	Cytotoxicity: no Genotoxicity: **negative**	Anonymous 31, 1996
***In vitro* chromosome aberration study in mammalian cells** With and without met. act. No guideline followed GLP Reliability 2 (according to the registration dossier)	**1-nitropropane** Purity: > 99 % Vehicle: DMSO	Chinese Hamster lung (CHL) cells Test conc.: 6-hour treatment without S9 : 625, 1250, 2500 and 5000 µg/mL 24- and 48-hour treatment without S9: 312.5, 625, 1250 and 5000 µg/mL 6-hour treatment with S9: 156.25, 312.5, 625, 1250, 2500 and 5000 µg/mL	Cytotoxicity: yes Genotoxicity: **negative**	Anonymous 32, 1994

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
***In vitro* DNA damage and/or repair study** OECD TG 482 Not GLP Reliability 2 (according to the registration dossier, however not enough information available to the DS, no access to raw data)	1-**nitropropane** Purity: 97.4 % Impurity: 2-nitropropane (2.3 %)	Primary hepatocytes from male and female Wistar rats Test concentrations: 0.1-10 mM	Cytotoxicity: no information available Genotoxicity: **negative**	Andrae *et al.*, 1988
***In vitro* gene mutation test in mammalian cells** OECD TG 476 Not GLP Reliability 2 (according to the registration dossier, however only summary available, not enough information to confirm the validity of the study)	**1-nitropropane** Purity: 97.4 % Impurity: 2-nitropropane (2.3 %)	Chinese hamster lung cells (V79) Test conc.: 0, 0.3, 1, 3, 6 and 10 mM	Cytotoxicity: yes Genotoxicity: **positive**	Roscher *et al.*, 1990
***In vitro* micronucleus test in mammalian cells** OECD TG 487 (Not GLP Reliability 2 (according to the registration dossier, however only summary available, not enough information to confirm the validity of the study)	**1-nitropropane** Purity: 97.4 % Impurity: 2-nitropropane (2.3 %)	Chinese hamster lung cells (V79) Test conc.: 0, 0.3, 1, 3, 6 and 10 mM	Cytotoxicity: yes Genotoxicity: **positive**	Roscher *et al.*, 1990
***In vitro* DNA damage and/or repair study** OECD TG 476	**1-nitropropane** Purity: 97.4 % Impurity: 2-nitropropane (2.3	Cell lines of extrahepatic origin, derived from rat (embryonic fibroblasts and carcinoma Walker rat), mouse (embryonic fibroblasts), hamster (fibroblasts lung and fibroblasts ovary) and man (embryonic fibroblasts lung, adenocarcinoma lung,	Cytotoxicity: unspecified Genotoxicity: **negative**	Andrae U. *et al.*, 1988

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Not GLP Reliability 2 (according to the registration dossier, however only summary available, not enough information to confirm the validity of the study)	%)	adenocarcinoma lung and epiderm. carcinoma larynx) Test concentrations: 0.1 – 10 mM		
***In vitro* gene mutation test in bacteria** With and without met. act. OECD TG 471 GLP Reliability 1 (according to the registration dossier)	**1-nitropropane** Purity: ~99 % Vehicle: DMSO	*S. typh* TA98, TA100, TA1535 and TA1537 and *E. Coli* WP2uvrA- Test conc.: 0, 8, 40, 200, 1000 and 5000 µg/plate (first experiment) and 0, 312.5, 625, 1250, 2500 and 5000 µg/plate	Cytotoxicity: no Genotoxicity: **negative**	Anonymous 33, 1994
***In vitro* gene mutation test in bacteria** With and without met. act. OECD TG 471 Not GLP Reliability 1 (according to the registration dossier, however not enough information to confirm the validity of the study)	**1-nitropropane** Purity: 97 % Vehicle: DMSO	*S. typh.* TA98, TA100, TA1535 and TA1537 Conc.: 0, 100, 333, 1000, 3333 and 10000 µg/plate	Cytotoxicity: no Genotoxicity: **negative**	Haworth S. *et al*., 1983

Table 22: 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
NITROETHANE				

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
***In vivo* micronucleus test** Prior to OECD TG 474 Prior to GLP CD-1 mice (Charles River) 14/sex/ in control groups 8/sex/dose Reliability 2 (according to the registration dossier, however study not available to the DS)	**Nitroethane**	Oral (gavage) 2x/day Doses: 0.25, 0.5 or 1.00 mL/kg bw/d (highest dose = half the oral LD50 value) Sacrifice 6h after the last dose Vehicle: unknown Concurrent control: tap water Positive control: methylmethanesulfonate (90 mg/kg bw/d, i.p. route)	No significant increase in the frequency of micronucleated polychromatic erythrocytes, at doses up to 1 mL/kg bw/d, in either sex. **Negative**	Hite *et al.,* 1979
NITROMETHANE				
***In vivo* micronucleus test in NCEs of B6C3F1 mice** OECD TG 474 Non-GLP 10 males + 10 females Inhalation Reliability 2 (according to the registration dossier)	**Nitromethane** Purity unknown	Treatment: ➢ 6 h/d ➢ 5 d/w for 13 weeks Test conc.: 94, 188, 375, 750 and 1500 ppm (= limit dose) Vehicle: not specified	No increase in the frequency of micronucleated erythrocytes was observed in the peripheral blood of male or female mice that had been administered nitromethane by inhalation for 13 weeks at concentrations up to 1500 ppm. **Negative**	NTP, 1997
		Due to very poor quality of the copy, the study will not be presented in the CLH report and will not be assessed.		Gocke *et al.*, 1981
1-NITROPROPANE				
***In vivo* micronucleus test** No guideline followed	**1-nitropropane** Purity: unspecified	Male SD rats (4-8/groups) Gavage	Genotoxicity: **negative** in the bone marrow, however positive in liver	George *et al.*, 1989

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Not GLP Reliability 2 (according to the registration dossier, however DS not access to raw data)		Single dose Bone marrow: 24 h: 100, 200, 300 and 400 mg/kg; 48h: 100, 200 and 300 mg/kg Liver: 72 h: 300 mg/kg (lethality observed at 500 mg/kg)	Toxicity: yes lethality at 500 mg/kg bw	
***In vivo* mammalian cell study : DNA damage and/or repair** No guideline followed Not GLP Reliability 2 (according to the registration dossier, however DS not access to raw data)	**1-nitropropane** Purity: 97.4 % Vehicle: olive oil	Wistar rats 9 controls and 2/sex after 1 h and 17 h IP, single injection Conc.: 20, 40, 60 and 80 mg/kg	Genotoxicity: **negative**	Andrae *et al.*, 1988
***In vivo* mammalian somatic cell study: cytogenicity/erythrocyte micronucleus** No guideline followed GLP compliance unspecified Reliability 2 (according to the registration dossier, however poor quality of the PDF file, difficult to analyse the data)	**1-nitropropane** Purity: unspecified	Mouse (5/sex/group) IP, single dose Conc.: no information available	Genotoxicity: **negative**	Kliesch and Adler, 1987

No human data available

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

***In vitro* data on Nitroethane**

In an *in vitro* gene mutation test (Mortelmans *et al.,* 1986), 4 bacterial *S. typh.* strains (TA98, TA100, TA1535 and TA1537) were exposed to nitroethane at doses of either 100, 333.3, 1000, 3333.3 or 10 000 µg/plate. No cytotoxicity was seen in any plate, except at the highest dose, in all strains. Precipitation was observed in the highest concentration tested in most experiments in all the strains. In all strains, the positive control compounds induced a clear increase in the number of revertants, both in absence and in presence of S9 metabolic fraction. No significant increase in the frequency of revertant colonies was observed for any of the bacterial strains with any dose (up to 10 mg/plate) either in presence or in absence of S9 metabolic fraction. Under the test conditions, the compound is therefore considered as non-mutagenic.

Table 23: Ames test results

Dose level (µg/plate)		0	100	333.3	1000	3333	10000	Positive Control
TA100	-S9	119 ± 2.1	109 ± 8.5	115 ± 1.2	99 ± 5.9	122 ± 3.5	116 ± 11.3	402 ± 44.8
	+ 10 % hamster S9	103 ± 3.8	87 ± 12.2	86 ± 3.7	87 ± 8.5	97 ± 11.5	105 ± 4.8	973 ± 88.4
	+ 10 % rat S9	101 ± 8.7	127 ± 7.3	114 ± 10.3	114 ± 5.5	122 ± 6.9	138 ± 1.8	800 ± 18.5
TA1535	-S9	11 ± 1.2	16 ± 0.7	15 ± 1.0	14 ± 2.4	19 ± 3.2	16 ± 2.7	135 ± 18.0
	+ 10 % hamster S9	8 ± 2.0	7 ± 1.5	6 ± 1.5	4 ± 2.0	9 ± 2.1	7 ± 0.9	325 ± 10.4
	+ 10 % rat S9	5 ± 0.9	10 ± 3.5	7 ± 1.3	15 ± 8.6	8 ± 0.9	8 ± 0.6	277 ± 26.0
TA1537	-S9	5 ± 1.9	10 ± 2.0	8 ± 2.2	8 ± 1.2	8 ± 1.0	8 ± 1.5	131 ± 13.5
	+ 10 % hamster S9	4 ± 0.6	5 ± 0.9	3 ± 0.9	4 ± 0.9	3 ± 0.9	4 ± 1.2	233 ± 3.3
	+ 10 % rat S9	6 ± 1.8	5 ± 1.0	8 ± 1.3	4 ± 1.8	4 ± 1.0	4 ± 0.9	136 ± 5.0
TA98	-S9	43 ± 3.6	31 ± 1.2	34 ± 1.3	32 ± 2.6	32 ± 1.3	38 ± 3.8	543 ± 68.0
	+ 10 % hamster S9	32 ± 4.6	27 ± 1.5	26 ± 5.2	33 ± 7.5	28 ± 6.7	31 ± 7.8	560 ± 10.0
	+ 10 % rat S9	32 ± 3.2	41 ± 6.5	32 ± 6.0	37 ± 4.7	39 ± 5.5	28 ± 4.2	199 ± 20.3

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

In another *in vitro* gene mutation test in bacteria (Anonymous 29, 1980), 5 strains of *S. typh.* (TA98, TA100, TA1535, TA1537 and TA1538) were exposed to vapours of nitroethane. A concentration of 55450 ppm caused cytotoxicity in strains TA1535 and TA1537 and therefore a concentration of 27725 ppm was tested. No significant increase was observed in the frequency of revertant colonies at a concentration of 27725 ppm in any of the bacterial strains either in presence or in absence of S9 metabolic fraction. As the highest non-cytotoxic concentration did not cause mutagenicity, no additional concentrations were tested to investigate the concentration-response relationship.

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

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

Table 24: CHO cells survival (N colonies/plate) after exposure to 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
		2	143	140	113	143	104	121	168	143	117	146
		3	123	138	133	131	130	122	140	164	123	124
	Avg. RCS (%)		100	102.9	89.4	99.5	85.5	89.6	106	108.2	86	96.6

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

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

Table 25: Mutation assay results (without S9), results in duplicate, in the initial test

Dose (µg/mL)	Assay	**Mutation result**	**Cloning efficiency (CE)**				**Mutants per million clonable cells**
		Total mutant colonies/plate	Test 1	Test 2	Test 3	CE (%)	
0	1	1	166	150	162	79.7	0.6
	2	7	154	138	127	69.8	5.0
46.9	1	20	107	108	124	56.5	17.7
	2	11	146	138	131	69.2	8.0
93.9	1	18	119	117	133	61.5	14.6
	2	11	101	120	128	58.2	9.5
187.8	1	30	104	108	112	54.0	27.8
	2	15	124	119	111	59.0	12.7
375.5	1	9	139	123	134	66.0	6.8

	2	13	144	116	160	70.0	9.3
751	1	8	97	117	103	52.8	7.6
	2	6	136	132	103	61.8	4.9
Positive control	1	210	69	61	82	35.3	297.2*
	2	235	62	82	91	39.2	300.0*

Table 26: Mutation assay results (with S9), in the initial test

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

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

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

Table 27: Mutation assay results (without S9), results in duplicate, in the confirmatory test

Dose (µg/mL)	Assay	**Mutation result**	**Cloning efficiency (CE)**				**Mutants per million clonable cells**
		Total mutant colonies/plate	Test 1	Test 2	Test 3	CE (%)	

0	1	2	176	168	178	87.0	1.3
	2	4	192	207	203	100.3	2.5
46.9	1	6	191	210	217	103.0	3.6
	2	2	160	184	170	85.7	1.3
93.9	1	19	214	208	229	108.5	8.8
	2	20	208	196	187	98.5	11.3
187.8	1	9	230	221	199	108.3	4.2
	2	6	257	215	246	119.7	2.8
375.5	1	9	193	195	-	97.0	5.2
	2	4	152	186	197	89.2	2.8
751	1	10	202	188	190	96.7	5.2
	2	19	187	183	170	90.0	11.7
Positive control	1	132	81	84	82	41.2	160.3
	2	160	94	93	104	48.5	164.9

Table 28: Mutation assay results (with S9) in the confirmatory test

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

Positive control B	1	287	169	173	165	84.5	169.8*
	2	299	162	141	131	72.3	206.7*

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

Table 29: HCD for mutant frequency in CHO cells (2007-2012)

Year	S9	Number	Range
2007	-	32	0.7-14.5
	+	32	1.3-32.2
2008	-	16	2.2-26.0
	+	15	2.3-24.2
2009	-	12	2.9-15.1
	+	12	3.4-15.6
2010	-	44	1.6-15.2
	+	46	1.6-14.3
2011	-	8	1.5-11.8
	+	8	0.0-10.3
2012	-	4	4.2-11.0
	+	4	5.8-9.1

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

In an *in vitro* gene mutation study in bacteria (Dayal *et al*., 1989), 3 strains of *S. typh.* (TA98, TA100 and TA102) were exposed to nitroethane at concentrations under 200 µmol/plate. Nitroethane was negative in the *in vitro* gene mutation tests but they were only performed in 3 bacterial strains and in absence of S9 metabolic fraction. It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations cells have actually been exposed. However, in the same study, 2-nitropropane induced a positive result at a low concentration (20 µmol/plate) suggesting that the test material remained in solution.

***In 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 30: Ames test results

Dose level (µg/plate)		0	100	333.3	1000	3333.3	10000	Positive Control
TA100	-S9	82 ± 2.8	104 ± 2.2	106 ± 10.3	92 ± 4.5	101 ±11.3	127 ± 9.1	461 ± 5.9
	+ 10 % hamster S9	104 ± 6.8	113 ± 7.5	111 ± 0.6	101 ± 8.7	105 ± 10.0	120 + 3.2	1720 ± 67.7
	+ 10 % rat S9	101 ± 6.1	109 ± 11.0	89 ± 4.7	94 ± 5.5	101 ± 8.4	99 ± 6.1	577 ± 26.1
TA1535	-S9	23 ± 2.0	19 ± 2.6	19 ± 1.3	21 ± 2.0	20 ± 3.0	23 ± 1.5	458 ± 19.8
	+ 10 % hamster S9	11 ± 1.5	10 ± 2.8	10 ± 1.5	11 ± 3.2	12 ± 1.8	14 ± 3.1	421 ± 16.5
	+ 10 % rat S9	9 ± 1.2	13 ±2.8	13 ± 2.1	9 ± 2.0	10 ± 1.9	14 ± 1.3	392 ± 23.1
TA1537	-S9	8 ± 2.6	7 ± 0.9	7 ± 1.2	8 ± 1.0	9 ± 1.7	7 ± 3.0	431 ± 20.9
	+ 10 % hamster S9	11 ± 0.9	13 ± 2.6	12 ± 3.2	13 ± 2.6	15 ± 2.1	12 ± 1.9	510 ± 10.7
	+ 10 % rat S9	12 ± 2.2	4 ± 1.5	4 ± 1.5	5 ± 0.3	3 ± 0.6	2 ± 0.6	221 ± 31.0
TA98	-S9	28 ± 1.5	37 ± 0.3	34 ± 4.3	31 ± 2.8	25 ± 2.6	30 ± 5.2	777 ± 23.2
	+ 10 % hamster S9	40 ± 1.9	43 ± 6.2	33 ± 5.6	44 ± 1.3	41 ± 0.9	36 ± 5.7	1598 ± 76.2
	+ 10 % rat S9	48 ± 4.3	48 ± 3.6	43 ±2.0	47 ± 4.5	37 ± 3.1	39 ± 1.2	511 ± 35.6

As a remark, it can be stated that it is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations bacteria have actually been exposed. Furthermore, while the test was run in triplicate, it is specified in Mortelmans *et al.* (1986) that only the last experimental results are presented in the article. However, DS would like to highlight the fact that data was reported as mean ± SEM, which raises questions such as: is it the mean of the triplicates? From which data was this mean calculated?

In another *in vitro* gene mutation test in bacteria (Anonymous 27, 1980), no significant increase was observed in the frequency of revertant colonies at a concentration of 23732 ppm in any of the bacterial strains either in presence or in absence of S9 metabolic fraction. As the highest non-cytotoxic concentration did not cause mutagenicity, no additional concentrations were tested to investigate the concentration-response relationship.

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

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

Table 31: Chomosomal aberration in CHO cells

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

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

Table 32: SCE assay results in CHO cells

Dose level (µg/mL)		N cells	N chrom	N SCEs	SCE/chrom	Rel. change of SCE/chrom (%)[a]
Without S9						
Nitromethane	497	50	1049	374	0.35	7.06
	1655	50	1049	394	0.37	12.79
	4965	50	1052	411	0.39	17.32
Distilled water	/	50	1048	349	0.33	/
Mitomycin-C	0.001	50	1050	534	0.50	52.72
	0.004	10	209	186	0.88	167.24
With S9						

Nitromethane	497	50	1050	407	0.38	-4.64
	1655	50	1052	383	0.36	-10.43
	4965	50	1051	381	0.36	-10.881
Distilled water	/	50	1053	428	0.40	/
Cyclophosphamide	0.125	50	1051	647	0.61	51.46
	0.500	10	210	241	1.14	182.35

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

In an *in vitro* gene mutation study in bacteria (Anonymous 28, 1975), results have to be taken with caution. Although not performed according to OECD TG 471, the overall quality of the test could be acceptable (dose-range finding, concurrent positive and negative controls, with and without metabolic activation,…), however, the compound was not soluble under treatment conditions, and consequently, it is not clear to which concentrations cells have been exposed. Furthermore, no specific measures were taken to ensure exposure to volatile compounds. There is also some ambiguity related to the reporting of the results obtained with the suspension test in TA1537 (swaps in reported results tables). The study was therefore disregarded due to poor data reporting.

In an *in vitro* gene mutation study in bacteria (Dayal *et al.*, 1989), nitromethane did not induce gene mutations in the absence of S9 mix, on 3 different strains of bacteria (TA98, TA100 and TA102). It is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations cells have actually been exposed. However, 2-nitropropane induced a positive result in the same study at a low concentration (20 µmol/plate) suggesting that test material remained in the solution. As the reporting data are poorly reported, the study should nevertheless be interpreted with caution.

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

Table 33: SHE cells transformation test results

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

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

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

In an *in vitro* micronucleus test in SHE cells (Gibson *et al.*, 1997), nitromethane was incubated with SHE cells, the doses depending of the vehicle: 0 (DMSO), 5.0, 5.5 and 6.0 µg/mL and 0 (media), 3500, 4000, 5000 µg/mL. In each dose group, an assessment of the percentage of binucleated cells and of the number of micronucleated cells was performed on 500 cells and 1000 binucleated cells, respectively. Only micronuclei that were non-

refractile, completely in the cytoplasm, distinctly separated from the nucleus, and that measured less that 33 % of the nucleus were taken into account. The test results were negative, with either vehicle.

Table 34: 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 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 35).

Table 35: 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 36)

Table 36: 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: mitomycine C; CP: cyclophosphamide

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

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

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

1-Nitropropane induced an up to 5-fold increase in repair incorporation in hepatocytes from male and female rats. However, the authors reported that this repair induction was attributed to 2-nitropropane that was present as an impurity (2.3 %).

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

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

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

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

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

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

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

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

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

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

No significant increase in the frequency of revertant colonies was observed for any of the bacterial strains with any concentration in two independent experiments either in presence or in absence of S9 metabolic fraction. (see Table 37 and Table 38)

Table 37: Number of revertants (number of colonies/plate) (experiment 1)

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

Table 38: Number of revertants (number of colonies/plate) (experiment 2)

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

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

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

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

No significant increase in the frequency of revertant colonies was observed for any of the bacterial strains with any concentration either in presence or in absence of S9 metabolic fraction.

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

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

***In 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 39: 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 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 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 40).

Table 40: Incidence of micronuclei and PCE

Experiment	A									B			
Sampling time	24 h					48 h				24 h			
Dose (in mg/kg)	0	100	200	300	PC	0	100	200	300	0	300	400	PC
Nb. animals tested	6	6	6	6	4	6	6	6	6	3	5	5	3
MN PCE/1000 PCE	0.83	1.00	1.42	1.58[A]	8.40[A]	0.92	1.17	1.08	1.83	1.33	1.70	1.50	8.33[A]
% PCE	34.0	30.6	31.4	28.1	24.7	39.9	33.4	34.4	28.0	39.1	44.1	43.4	35.8

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

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

Nitropropane was negative in the *in vivo* micronucleus test in bone marrow but induced an increase in the micronuclei frequency in hepatocytes which was assigned to increased cell proliferation.

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

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

The article mentions that "the test substance did not cause increase repair synthesis in males treated with 20 – 80 mg/kg for 4 h but did slightly reduce the repair background. Likewise, no repair induction was observed when male rats were injected with 60 mg/kg and killed 1 h or 17 h later. 1-nitropropane was also ineffective in inducing repair in HPC from female rats treated *in vivo*"

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

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

10.8.2 Comparison with the CLP criteria

CLP criteria cat. 1	CLP criteria cat. 2
Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans.	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans.

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

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

A non significant increase in the number of 6-thioguanine resistant mutations was observed in Chinese Hamster lung cells V79 after treatment with 1-nitropropane (Roscher *et al.*, 1990). However, it is not clear whether the protocol was adapted for volatile compounds and consequently, it remains unknown to which concentrations cells have actually been exposed.

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

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

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

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

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

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

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

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

Table 41: Summary data regarding *in vitro* tests

In vitro				
Test Guidelines	**Substances**	**Results**	**References**	**Remarks**
OECD TG 471	NM	Negative	Mortelmans *et al.*, 1986	/
	NM	Negative without S9	Dayal *et al.*, 1989	/
	NM	Negative	Anonymous 27, 1980	Prior to an OECD TG 471 test
	NM	-	Anonymous 28, 1975	Prior to an OECD TG 471 test Disregarded due to poor data reporting + test material not soluble under the treatment conditions
	NE	Negative	Mortelmans *et al.*, 1986	/
	NE	Negative without S9	Dayal *et al.*, 1989	/
	NE	Negative	Anonymous 29, 1980	Prior to an OECD TG 471 test
	1-NP	Negative	Anonymous 31, 1996	/

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

Table 42: Summary data regarding *in vivo* tests

In vivo				
Test Guidelines	**Substances**	**Results**	**References**	**Remarks**
OECD TG 474	NM	Negative	NTP, 1997	/
	NE	Negative	Hite and Skeggs, 1979	/
No guideline micronucleus test	1-NP	Negative in the bone marrow Positive in the liver	George *et al.*, 1989	/
No guideline mammalian cell study : DNA damage and/or repair	1-NP	Negative	Andrae *et al.*, 1988	/
No guideline mammalian somatic cell study: cytogenicity/erythrocyte micronucleus	1-NP	Negative	Kliesch and Adler, 1987	/

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

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

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

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

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

Consequently, data is considered inconclusive for germ cell mutagenicity.

10.9 Carcinogenicity

Table 43: 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
NITROETHANE			
Long term inhalation toxicity study 2 years Similar to OECD TG 453 GLP compliant: not specified Rat Long-Evans 40/group (control & 100 ppm) 41 males & 39 females (200 ppm) Reliability 2 (according to the registration dossier) Major deviations: - only 2 doses tested - 40 animals / group - some tissues were not examined microscopically (parathyroid, caecum, rectum, bone marrow,…)	**Nitroethane** Purity: 97.92 % Impurities: nitromethane 0.01 % and 2-nitropropane 2.07 % Inhalation 7 h/d, 5 d/w Conc.: 0, 100, 200 ppm (corresp. approx. to 0, 0.31 and 0.61 mg/L, resp.)	Mortality: no treatment-related effect BW: sign. ↓ at 100 ppm in males and at 200 ppm in females Clinical chemistry: slight but sign. ↑ of tot. prot. and BUN in females exposed to 200 ppm Hematology: No effects observed. MetHb levels not assessed. Organ weights (brain, liver, kidneys, lungs, heart): no treatment-related effect Histopathology: no effect Neoplastic effects: - No treatment-related increase of tumours - In all animals (controls and treated groups), high incidence of benign tumours (adenoma of the pituitary gland) - Very rare malign tumours, not treatment-related - No HCD available	Anonymous 35, 1986
NITROMETHANE			

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Long term inhalation study Similar to OECD TG 451 GLP-compliant 2 years Rats, F344/N 50/sex/dose Reliability 1 (according to the registration dossier)	**Nitromethane** Purity: > 99 % inhalation 6 h/d, 5 d/w 0, 94, 188, 375 ppm (approx. equivalent to 0, 0.235, 0.47 and 0.94 mg/L, resp.)	Mortality: relatively high in all groups but not dose-related (74, 68, 72 and 84 % in males and 44, 62, 40 and 54 % in females at 0, 94, 188 and 375 ppm, resp.) Clinical signs: masses on shoulders and torso consistent with mammary gland neoplasms BWG: slightly increased in females exposed to 375 ppm vs. controls Organ weight: no data Histopathology: - In males: hyperplasia in renal tubule (6, 8, 6 and 12 out of 50 males, at 0, 94, 188 and 375 ppm, resp.) - In females: mammary gland fibroadenoma, fibroadenoma or adenoma (combined) and fibroadenoma, adenoma or carcinoma (combined) increased in a dose-dependent manner (see below) Neoplastic effects: In females: **Mammary gland**, out of 50 animals and at 0, 94, 188 and 375, resp. (%): - 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
Long term inhalation study Equivalent or similar to OECD TG 451 GLP-compliant 2 years Mice,	**Nitromethane** Purity: > 99 % Impurities: 0.25 % nitroethane, 0.03 % 2-nitropropane inhalation	Mortality: 38, 28, 40 and 42 % of males and 50, 44, 48 and 28 % of females exposed to 0, 188, 375 and 750 ppm, resp., died Clinical sign: in the eyes, swelling and exophthalmos coincident with harderian gland tumours, in both sexes BWG: no effects in males, slightly increased BW in females during the study but similar to controls at study termination	NTP, 1997

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
B6C3F1 50/sex/group Reliability 1 (according to the registration dossier)	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.)	Organ weights: no data Histopathology: - Sign. increased incidence olfactory epithelium degeneration in both sexes, in all treated groups - Sign. increase in olfactory epithelium metaplasia in both sexes at 375 and 750 ppm - Sign. increase in respiratory epithelium hyaline degeneration in all treated groups in females and at the middle and high doses in males. Neoplastic effects **- Harderian gland**: Male and female: Adenoma (%): M: 9/50 (18), 10/50 (20), 19/50 (38)**, 32/50 (64)** (HCD: 2-14 %) F: 5/50 (10), 7/50 (14), 16/50 (32)**, 19/50 (38)** (HCD: 0-16 %) Carcinoma (%): M: 1/50(2), 1/50 (2), 6/50 (12), 5/50 (10) (HCD: 0-4 %) F: 1/50 (2), 2/50 (4), 4/50 (8), 3/50 (6) (HCD: 0-4 %) Adenoma or carcinoma (%): M: 10/5 (20), 11/50 (22), 25/50 (50)**, 37/50 (74)** (HCD: 2-14 %) F: 6/50 (12), 9/50 (18), 20/50 (40)**, 21/50 (42)** (HCD: 0-16 %) **- Liver**: Female (%): Hepatocellular adenoma: F: 14/50 (28), 25/49 (51)*, 17/49 (35), 35/50 (70)** (HCD: 0-40 %) Hepatocellular carcinoma: F: 10/50 (20), 14/49 (29), 8/49 (16), 12/50 (24) (HCD: 2-30 %) Hepatocellular adenoma or carcinoma:	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		F: 19/50 (48), 34/49 (69)**, 22/49 (45), 40/50 (80)** (HCD: 6-54 %) No increase in liver tumours was observed in Males. **Lung:** Male and female (%): Alveolar/bronchiolar adenoma: M: 11/50 (22), 10/50 (20), 9/50 (18), 12/50 (24) (HCD: 6-36 %) F: 3/50 (6), 3/50 (6), 2/49 (4), 9/50 (18) (HCD: 0-14 %) Alveolar/bronchiolar carcinoma: M: 2/50 (4), 3/50 (6), 3/50 (6), 11/50 (22)** (HCD: 0-16 %) F: 0/50 (0), 3/50 (6), 5/49 (10), 3/50 (6) (HCD: 0-6 %) Alveolar/bronchiolar adenoma or carcinoma: M: 13/50 (26), 13/50 (26), 12/50 (24), 20/50 (40) (HCD: 10-42 %) F: 3/50 (6), 6/50 (12), 6/49 (12), 12/50 (24)* (HCD: 0-16 %)	
Long term inhalation toxicity study Rats / Long-Evans / male + female 40 animals/group OECD TG 451 GLP not specified Reliability 1 (according to the registration dossier) Major deviations from OECD TG 451 guideline:	**Nitromethane** Purity: 96.26 % Impurities: 2.79 % nitroethane, 0.62 % 2-nitropropane Inhalation Doses: 0, 100, 200 ppm (approx. equivalent to 0, 0.25 and 0.50 mg/L, resp.) Duration of exposure: 7 h/d, 5 d/w for 103 w	Mortality: 37.5, 42.5 and 37.5 % of males and 25, 27.5 and 40 % of females died Body weights: - similar to controls in males, - sign. lower than controls in females after 1 year exposure at 100 and 200 ppm Clinical chemistry: no clinically significant effects in either sex Hematology: no effects in either sex Organ weights (brain, liver, kidneys, lungs, heart): no effects in relative and absolute weights, in both sexes Histopathology: effects were observed in all animals (controls + exposed) but were not treatment-related: bronchitis, glomerulosclerosis, calcification of the kidneys, vacuolation of the adrenal cortex and fibrocystomas in the mammary gland. Neoplastic effects:	Anonymous 34, 1990

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
- only 2 doses were tested - 40 animals / group - some tissues were not examined microscopically (parathyroid, epididymis, caecum, rectum, bone marrow,…)		- 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.	
1-NITROPROPANE			
Long term inhalation toxicity study Rat / Long Evans / male + female 125/sex (10/sex/group and the remaining alive were killed after 21.5 months of exposure) No guideline followed GLP compliance: unspecified Reliability 2 (according to the registration dossier)	**1-nitropropane** Purity: unspecified Doses: 0 or 100 ppm, approx. equivalent to 0 and 0.369 mg/L, resp Duration of exposure: 1, 3, 12, 18 and 21.5 months + 2 additional groups: exposed during 21.5 months and thereafter observed during 3 months or 12 months	Mortality: increased in treated groups Clinical signs: not specified Body weight: inconsistent differences, no treatment-related effects Organ weight: no treatment-related changes (brain, kidneys, liver examined) Histopathology: few incidences of liver vacuolization and a number of parenchymal abscesses in animals found dead Benign tumours: increased incidence of pituitary adenoma after 18m of exposure (in control and treated groups) Malignant tumours: slightly increased incidence of lymphosarcoma in spleen and lymph nodes in animals found dead in control and treated groups	Griffin *et al.*, 1982
Assay of 1-nitropropane, 2-nitropropane, 1-azoxypropane and 2-azoxypropane for carcinogenicity Rat / SD / male Nb of animals not specified	**1-nitropropane** Doses: 0 and 89.1 mg/kg bw 3 times/week for 16 w followed by 1 time/w for 10 w Duration of exposure:	Body weight and necropsy findings: treatment-related effects observed (no more information available) No increase of tumour incidence (no more detail given)	Fiala *et al.,* 1987

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Gavage No guideline followed Not-GLP Reliability 2 (according to the registration dossier, however only summary available to the DS)	26 w Surviving animals were sacrificed after 77 w		
Test for chemical carcinogens Rat / F344 / both sexes Nb 3/sex/dose except at the mid-dose (15/sex) Gavage No guideline reported Not-GLP No access to raw data, not reported in the registration dossier	**1-nitropropane** Doses: 0, 0.3, 3 or 10 mg/d 5 times/week, for 52 weeks	No increase in tumour incidence	Hadidian *et al*., 1968

No human data or other relevant information available.

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

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 44, at least 50 % of the control group did not survive during the 2-year study. No historical control data is available.

Table 44: 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 45: 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 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 46: 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 47: 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 48), confirming clinical observations and possibly explaining the increase in body weights at higher doses.

Table 48: 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					
Females	Adenoma (%)	2/50 (4)	0/50	0/50	2/50 (4)	3/348 (0.9 ± 1.6 %) 0-4 %
	Fibroadenoma (%)	19/50 (38)	21/50 (42)	33/50** (66)	36/50** (72)	97/348 (27.9 ± 7.3 %) 20-40 %
	Carcinoma (%)	2/50 (4)	7/50 (14)	1/50 (2)	11/50** (22)	14/348 (4 ± 2.6 %) 0-8 %
	Adenoma, fibroadenoma and carcinoma (%)	21/50 (42)	25/50 (50)	35/50** (70)	41/50** (82)	108/348 (30.9 ± 9.1 %) 22-46 %

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

In female rats, the incidence of fibroadenoma, fibroadenoma or adenoma, and fibroadenoma, adenoma or carcinoma was dose-dependent and incidences at the middle and high doses were statistically significant. The tumours incidence in the low, mid and high dose groups were outside the range of the historical control data,

whereas, incidence in control group was included in these ranges. Carcinomas tended to appead earlier in treated groups, compared to the control group.

Table 49: 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 50: 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 approximatively equivalent to 0, 0.47, 0.94 and 1.87 mg/L, respectively. Mortality tended to be high in all dose groups (see Table 51), in both sexes, but the survival rate of females exposed to the highest dose was marginally greater than in other groups. Coincidently with a swelling around the eyes and exophthalmos in exposed animals of both sexes, neoplasms of the Harderian gland were observed (see Table 54 below). Nasal lesions were reported in a great number of exposed animals of both sexes (see Table 53). Tumours incidence in the Harderian gland, the liver and the lung are presented in Table 54 below. Liver tumours were seen only in females.

Table 51: 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 52: 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 53: 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 54: 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 %) 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 55: 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 56: 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 57: 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 58: 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 59 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 59: 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, Hg, Hct, 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 60: Tumours incidence

Dose level (ppm)		**0**	**100**	**200**
In males				
Mammary gland	**Adenocarcinoma**	0	2	0
	Fibroadenoma	0	1	0
	Fibroma	0	0	1
	Cystadenoma	0	0	1
	Adenoma	14	14	15
Pituitary gland	Adenoma C-cell	2	4	3
Thyroid	Adenocarcinoma	0	2	0
Liver	Metastasis primary mesenchymal	1	1	3
In females				
Mammary gland	Fibroadenoma	7	8	14
	Multiple fibroadenoma	9	2	3
	Adenocarcinoma	3	0	2
Uterus	Adenoma			
	Adenonocarcinoma	0	0	1
	Myosarcoma	1	0	1
Thyroid	Adenoma C-cell	1	0	2
Pituitary gland	Adenoma	26	26	24

Liver	Meta. Primary mesenchymal	0	2	1

Malign tumours in bold

Data on 1-Nitropropane

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

Inconsistent differences were observed during the body weight and hematology examination (see Table 61 and Table 62). Necropsy did not reveal any treatment-related organ weight changes, and only infrequent findings were observed amongst control and exposed groups.

Table 61: 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 62: 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 63 and Table 64). 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 63 and 64).

Table 63: Incidence (inc.) of pituitary adenoma

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

Table 64: 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 65: 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 66: 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.

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

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

		fibroadenomas of the mammary 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 *et al.*, 1982	/
No guideline, carcinogenicity study	1-NP	No increase of tumour incidence	Fiala *et al.,* 1987	/
No guideline, Test for chemical carcinogens	1-NP	No increase in tumour incidence	Hadidian *et al*., 1968	/

Table 67: 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
NITROETHANE								
Rat (Long-Evans)	Pituitary adenoma Increase similar in controls No data on background incidence	No	No	/	Both	/	Inhalation	/
NITROMETHANE								
Rat (Long-Evans)	No treatment-related increase of tumours	/	/	/	/	/	Inhalation	/
Rat (F344)	**Mammary gland**: Adenoma, fibroadenoma or carcinoma	No	Yes	/	Only in females	/	Inhalation	Non-genotoxic but a positive result was obtained in the SHE transformation assay The concordance between the SHE assay and rodent bioassay is high. The mode of action has not been elucidated and therefore should be assumed relevant for humans
Mice (B6C3F1)	**Harderian gland** Adenoma or carcinoma.	Yes Tumours are observed in Harderian gland, lungs	Yes	-	both	No	Inhalation	No similar tissue is found in humans. The tissue is known to be sensitive to genotoxic compound but 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
		and liver						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	
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
Known or presumed human carcinogens A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as: Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: – human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or – animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with	Suspected human carcinogens The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited(1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

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

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

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

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

evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs."

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

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

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

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

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

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

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

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

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

Finally, a significant dose-dependant increase of malignant tumours of Harderian glands was observed in male and female mice but this tissue has no equivalent in humans. The observation of Harderian glands tumours in rodents is seen as an indication of the carcinogenic potential of the test-substance in the whole weigh-of-evidence analysis, especially when reported in association with other tumours (multi-site response). However, this tumour-type as such is considered not relevant to human.

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

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

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

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

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

10.9.3 Conclusion on classification and labelling for carcinogenicity

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

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

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 68: 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
NITROETHANE			
13-week inhalation toxicity study Similar to OECD TG 413 Mainly GLP Mouse B6C3F1 Male/female 15/sex/dose Reliability 2 (according to the registration dossier)	**Nitroethane** Purity: > 97 % Inhalation 6 h/d, 5 d/wk, 13 w 0, 100, 350, 1000 ppm equivalent to 0, 0.3, 1.0, 3.0 mg/L, resp.	**Parental toxicity:** No effect on BW, food consumption, clinical signs At 1000 ppm: Effects seen in the salivary glands, liver, and olfactory nasal epithelium At 350 ppm: Effects seen in liver, salivary glands and nasal turbinates and MetHb levels were affected At 100 ppm: Minimal changes reported (only in nasal turbinates and transiently in salivary gland epithelium) **Sexual function and fertility:** Sperm parameters not evaluated At 1000 ppm: Effects seen in the testes as significant increase of relative testicular weight and hyperplasia and multinucleated spermatids, effects in epididymes: at interim sacrifice slight focal unilateral decreased spermatogenesis in tubules (1/4 males), slight focal unilateral interstitial hyperplasia in testis (1/4) and slight focal mononuclear aggregates in epididymis (1/4); at terminal kill very slight multifocal bilateral multinucleated spermatids (1/5), slight multifoc. bilat. multinucleated spermatids (1/5) and very slight multifoc. bilat. multinucl. spermatids in tubules (1/5) In females at terminal kill: primary benign teratoma in ovary (1/5), very slight focal muscularis acute inflam. in cervix (1/5)	Anonymous 26, 1982

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		At 350 ppm: In testis, significant increase of relative testicular weight	
13-week inhalation toxicity study Similar to OECD TG 413 Mainly GLP Rat F344 15/sex/dose Reliability 2 (according to the registration dossier)	**Nitroethane** Purity: >97 % Inhalation 6 h/d, 5 d/wk, 13 w 0, 100, 350, 1000 ppm equivalent to 0, 0.3, 1.0, 3.0 mg/L, resp	**Parental toxicity:** Statistically significantly decreased body weight in the 350 ppm (D49 for males and D61 for females) and 1000 ppm exposure groups (D44 in males and D61 for females) Cyanotic color of the skin (visible at 350 ppm after 9 w of exposure and in 1000 ppm after 4 exposure), dull and dark red eyes (visible at 350 ppm after 4 w of exposure and in 1000 ppm after the first exposure only) in both sex, unkept appearance in females No neoplastic lesions found at necropsy Effects on several absolute and/or relative organ weights. **Sexual function and fertility:** Relative testes weights were increased in a statistically significant way, in the 350 and 1000 ppm groups, in comparison with the controls.	Anonymous 26, 1982
Disregarded study Teratology study in mice Reliability 4 (according to the registration dossier)	/	Co-exposure to 8.9 ± 2.0 ppm diethylhydroxylamine and 14.3 ± 2.0 ppm nitroethane from GD 6 to GD 17 for 8.25 ± 2.25 h/d, 5 d/w. furthermore, continuous exposure to diethylamine hydrogen sulfite 24/7 also occured.	Beliles *et al.,* 1978
Disregarded study 3-generation toxicity study Reliability 4 (according to the registration dossier)	/	Co-exposure to 7.8 ± 1.2 ppm diethylhydroxylamine and 11.5 ± 2.9 ppm nitroethane for 8.25 ± 2.25 h/d, 5 d/w. Furthermore, continuous exposure to diethylamine hydrogen sulfite 24/7 also occured.	Heicklen *et al.,* 1979
NITROMETHANE			
13-week repeated dose inhalation toxicity study	**Nitromethane** Purity: > 98 %	Mortality: / BW: Significant decrease in BW and BWG in males exposed to 1500 ppm	NTP, 1997

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
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)	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	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 significant change in the estrous cycle length significant decrease in sperm motility at 750 and 1500 ppm	
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) Reliability 2 (according to the DS)	**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
1-NITROPROPANE			
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Rat (SD) (Crl: CD(SD) IGSBR) 12/sex/dose OECD TG 422 GLP Reliability 1 (according to the registration	**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	**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 premating period Organ weight: in males at highest dose: signif. lower FBW and signif. higher relative brain and relative testes weights	Anonymous 37, 2003

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
dossier)	Duration of exposure: 14 d of premating period, during mating for both sexes and until gestation day 19 for females	**Sexual function and fertility** Reproductive performance: 2 females failed to become pregnant at the mid and high dose levels ***Developmental effects (assessed in sections 10.10.4-10.10.6)*** *Litter size: lower at the highest dose (not signif. however outside the range of HCD)* *Pup BW: significantly higher at 100 ppm in both sexes at lactation day 1 and 4 (within HCD range)*	

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 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 (Table 103) was observed, as well as an increase in methemoglobin levels (associated with cyaniosis), in reticulocytes and Heinz bodies in blood associated with splenic congestion and extramedullary hematopoiesis. Degenerative and inflammatory modifications were seen in nasal epithelium, vacuolization of hepatocytes, reduced cytoplasmic granularity of kidney cortical tubular epithelial tissue and ductal epithelial cells in the salivary glands. At the middle dose, same changes, although to a lesser intensity, were observed in methemoglobin levels, spleen, nasal epithelium and salivary glands. The changes were minimal at 100 ppm in the methemoglobin level, spleen and salivary glands. Growth retardation was reported in the 1000 and 350 ppm in female and male rats. All of these treatment groups had statistically significant body weight decreases when compared to controls during the last month of the study, despite the fact that the 1000 ppm female rats weighted statistically significantly less than their controls prior to the start of the study. Group mean body weight for both sexes of the 100 ppm group were comparable to their controls.

Two clinical findings, cyanosis and red eyes, were consistent with the grossly observable treatment-induced methemaglobinemia (Table 104).

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

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

- Female rats of the 100, 350 and 1000 ppm exposure groups had an unkept appearance which was an expression of their general weakened condition, secondary to the toxicity of the test material.

Two other clinical findings, swelling in the salivary gland region and increased amounts of porphyrin pigments around the nares, were observed in some rats of the 100, 350 or 1000 ppm group. These observations were consistent with a mild transient viral infection (sialodacryoadenitis) which commonly occured in this laboratory and were not judged to be treatment-related.

Prior to interim kill (20th exposure day, D29 of the experiment), methemoglobin was dosed in blood, 15 hours after the last exposure (Part A of Table 104). 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 104).

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 104).

At terminal kill, a time-sequenced analyse (Part D of Table 104) was performed less than 30 min after exposure, 4 and 19h after exposure in rats. 19-h after exposure, methemoglobinemia was similar in control, 100 and 350 ppm groups. The level was however significantly increased at 1000 ppm.

Prior to the interim kill (30 days), statistically significant lowered hemoglobin values in male rats and statistically significant increases of the WBC counts were seen in the 1000 ppm group. At 350 and 1000 ppm, increased levels of reticulocytes and Heinz bodies were noted.
Prior to the terminal kill (92 days), a statistically significant increased PCV and a decreased RBC count was noted in females as well as statistically significant lowered hemoglobin values in male rats, at 1000 ppm. At 350 and 1000 ppm, increased levels of reticulocytes and Heinz bodies were noted. (Table 104)

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

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

Dose level (in ppm)		0	100	350	1000
Body weight		229.8 +/- 13.5	219.6 +/- 9.3	216.6 +/- 8.3	203.8* +/- 9.3
Testes	Abs	2.92 +/- 0.17	2.75 +/- 0.06	2.80 +/- 0.08	2.81+/- 0.06
	Rel	1.27 +/- 0.04	1.25 +/- 0.06	1.29 +/- 0.03	1.38* +/- 0.05

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

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

Table 71: Histopathological observations

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

Slight hyperplasia in ducts	0	0	1	1
Methb (% ± St. Dev.)	0.5 ± 0.3	5.3 ± 1.7	30.7* ± 3.9	61.8* ± 6.0

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

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

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

Table 72: Testes weight at terminal kill

Dose level (in ppm)		0	100	350	1000
Body weight		34.3 +/- 2.0	33.6 +/- 2.5	32.4 +/- 2.6	32.4 +/- 2.5
Testes	Abs	0.22 +/- 0.02	0.22 +/- 0.02	0.23 +/- 0.02	0.23 +/- 0.02
	Rel	0.64 +/- 0.06	0.65 +/- 0.05	0.70* +/- 0.05	0.72* +/- 0.03

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

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

Table 74: Histopathological observations

Dose level (ppm)	0	1000
N examined	5	5
Males		
N testes tissues assessed	5	5
N Lesions testes	4	2
Testes degeneration	1 S.	0
Multinucleated spermatids, b., m.		1 V.S. 1 S.
Multinucleated spermatids tubules, b., m.	0	1 V.S.
N lesions epidydimis	5	5

N lesions seminal vesicle	5	5
N with affected prostate	5	5
N coagulated gland (examined/affected)	3/3	2/2
Females		
N ovary examined	5	5
N affected ovary	5	4
Benign teratoma, no meta., primary	0	1
N oviduct affected	5	5
N uterus (affected/examined)	4/5	4/5
N cervix (affected/examined)	4/4	4/5
Acute inflammation muscularis, focal	0	1 V.S.

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

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 75). Hindlimbs paralysis was reported in all animals (both sexes) exposed to 1500 ppm starting from D21 and in 1/10 male and 4/10 females exposed to 750 ppm, starting from D63. Hematology findings showed a dose-dependent microcytic responsive anemia (with decreased Hg concentration at all time points in all animals exposed to 375, 750 and 1500 ppm and at several time points at 94 and 188 ppm). No modifications were reported in organ weights.

Table 75: 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 76: Reproductive data

Exposure level (ppm)		0	375	750	1500
Males					
	N	10	10	10	10
Sperm parameters	Motility	94.57 ± 1.30	92.16 ± 1.90	87.11 ± 1.88**	76.43 ± 2.78**
	Count	64.33 ± 3.89	62.75 ± 3.63	62.68 ± 3.02	68.95 ±3.14
Weights (g)[a]	FBW at termination	338 ± 7	341 ± 4	331 ± 4	299 ± 11**
	L. cauda	0.207 ± 0.004	0.210 ± 0.004	0.204 ± 0.006	0.177 ± 0.009**
	L. epididymis	0.467 ± 0.009	0.468 ± 0.006	0.444 ± 0.009	0.412 ± 0.013**
	L. testis	1.39 ± 0.03	1.36 ± 0.01	1.34 ± 0.02	1.29 ± 0.02**
Females					
	N	10	10	10	10
Weight (g)	At termination	188 ± 5	200 ± 5	195 ± 4	178 ± 3
Estrous cycle length	In days	4.89 ± 0.07a	4.75 ± 0.16b	5.00 ± 0.14a	5.00 ± 0.15

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

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

Table 77: 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 interprete the adversity of the observed increased oestrus cycle length in females based on the available dataset without further investigation. The DS however highlights that this effect seems to be treatment-related as it is clearly dose-dependent and statistically significant at all doses.

Table 78: 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 79: Estrous cycle length

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

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

Data on 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 premating period (see Table 80). These change were not observed in females (see Table 81).

Table 80: 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 81: Body weight data in females (in g)

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

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

At necropsy, organ weight was examined. Males exposed to 100 ppm showed a significantly reduced final body weight value (354.1, 358.8, 357.3 and 328.7* g respectively at 0, 25, 50 and 100 ppm) as well as a significantly higher relative brain weight (0.562, 0.567, 0.572 and 0.622* g/100g respectively at 0, 25, 50 and 100 ppm) and relative testes weight (0.867, 0.902, 0.846 and 0.965* g/100g respectively at 0, 25, 50 and 100 ppm). Organ weights in females were not significantly changed. Histopathology examination revealed effects in females nasal tissue (such as multifocal degeneration of the olfactory epithelium, sometimes with signs of inflammation) (see Table 83).

Table 82: 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 83: Incidence of nasal tissue degeneration

		Males				Females			
Dose level (in ppm)		0	25	50	100	0	25	50	100
Nb of animal examined		12	12	12	12	12	12	12	12
Within normal limits		12	12	12	9	9	10	8	1
Degeneration of the olf. epith. (multifocal)	Very slight	0	0	0	1	0	0	0	5
	slight	0	0	0	1	0	0	0	2
Degeneration of the olf. epith. with inflammation (focal)	Very slight	0	0	0	0	0	0	2	0
Degeneration of the olf. epith. with inflammation (multifocal)	slight	0	0	0	0	0	0	0	2
Chronic inflammation of the epith. (squamous cell) (focal)	Very slight	0	0	0	0	2	1	0	0
	Slight	0	0	0	0	0	0	0	1
Chronic inflammation of the epith. (squamous cell) (multifocal)	Very slight	0	0	0	0	1	1	1	2
	slight	0	0	0	1	0	0	2	1

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

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

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

- Sperm parameters:

Sperm was examined in two studies performed with nitromethane. As observed in Table 84, these two studies revealed that sperm was affected by treatment. In the 13-week repeated dose inhalation toxicity study in rat (NTP, 1997), a significant and dose-dependent decrease in sperm motility was evidenced with 94.57, 92.16, 87.11** and 76.43** % at 0, 375, 750 and 1500 ppm. This was also reported in mice. Indeed, in the 13-week repeated dose inhalation toxicity study in mice (NTP, 1997), a significant decrease in sperm motility was observed with 93.5, 85.09**, 86.47** and 82.42** % at 0, 375, 750 and 1500 ppm, respectively. The decrease in sperm motility observed is considered treatment-related based on a dose-dependance and a statistical significance at mid and high dose in two different species. In addition, the absence of body weight loss in mid-dose animals indicates that the decreased sperm motility cannot be linked to unspecific systemic toxicity. It should be noted that these 13-week repeated dose inhalation toxicity studies are not reproductive toxicity studies, the study design therefore implies that the reproductive effects are moderate and cannot be associated with a potential decrease of the reproductive function (such as litter size or the number of pregnant dams). However, the effects were reported at dose level which also showed concentration-dependent microcytic responsive anemia. As reported in Reyes *et al.* study (2012), hypoxia can lead to adverse effects on spermatogenesis. Nevertheless, the article mentions that "*A reduced sperm count can be related to the increase in germ cell apoptosis promoted by this hypoxic condition. The same results were observed in male rhesus monkeys. Morphological studies have revealed that chronic hypoxia causes degeneration of the germinal epithelium, folding of the basement membrane, degeneration and detachment of germ cells, changes in lipid droplets in Sertoli cells, and an increase in lipoperoxidation. Other local changes in the testicles have also been observed, including an increase in vascularization, an increase in testicular temperature, a decrease in testicular mass, and an increase in interstitial space*". Other effects which were not observed in the available studies. The CLP guidance noted that "*Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary nonspecific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate*".

Sperm parameters were not examined in the available studies performed with 1-nitropropane or with nitroethane. However, in the combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonyous 37, 2003), two females exposed to 50 and 100 ppm failed to be pregnant, resulting in a fertility index of 100.0, 100.0, 83.3 and 83.3 %, resp. at 0, 25, 50 and 100 ppm. The reduction 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 84, male reproductive organ exhibited variation in different studies. Some of them were significant.

Table 84: Male fertility parameters

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

- Female reproductive organ:

In the 13-week repeated dose inhalation toxicity study in rat (NTP, 1997) performed with nitromethane, oestrous cycle length was not significantly affected. However in the same study performed in mice (NTP, 1997), it was signicantly and dose-related increased at the 3 tested doses (4.00, 4.33*, 4.50* and 4.71**, resp.

at 0, 375, 750 and 1500 ppm). No studies performed with nitroethane and 1-nitropropane examined the oestrous cycle length. As mentioned before, in the Combined Repeated Dose Toxicity with the Reproduction/Developmental Toxicity Screening Test (Anonyous 37, 2003), two females exposed to 50 and 100 ppm failed to be pregnant, resulting in a fertility index of 100.0, 100.0, 83.3 and 83.3 %, resp. at 0, 25, 50 and 100 ppm. The reduction 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 85: 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 86: 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
NITROETHANE			
Disregarded study Teratology study in mice Reliability 4 (according to the registration dossier)	/	Co-exposure to 8.9 ± 2.0 ppm diethylhydroxylamine and 14.3 ± 2.0 ppm nitroethane from GD 6 to GD 17 for 8.25 ± 2.25 h/d, 5 d/w. furthermore, continuous exposure to diethylamine hydrogen sulfite 24/7 also occured.	Beliles *et al.,* 1978
Disregarded study 3-generation toxicity study Reliability 4 (according to the registration dossier)	/	Co-exposure to 7.8 ± 1.2 ppm diethylhydroxylamine and 11.5 ± 2.9 ppm nitroethane for 8.25 ± 2.25 h/d, 5 d/w. Furthermore, continuous exposure to diethylamine hydrogen sulfite 24/7 also occured.	Heicklen *et al.,* 1979
NITROMETHANE			
Prenatal Developmental Toxicity Study Rat (Wistar) 24 females/group (2 females mated with 1 male) OECD TG 414 GLP Reliability 1 (according to the registration dossier) Deviations: identification of males via a subcutaneous transponder and not a mark on the tail, variation of the relative humidity from 44.9 to 65 % and no use of the surplus animals for training purpose.	**Nitromethane** Purity: > 99 % Inhalation (vapours) Doses: 0, 300, 600 and 1200 ppm (± 0, 0.75, 1.50 and 3 mg/L, resp.y) Duration of exposure: 6 h/d, from GD 6 to 20	Actual concentrations in chamber: 303, 601 and 1178 ppm (similar to 0.75, 1.50 and 2.99 mg/L, resp.) **Maternal toxicity:** Mortality: / Clinical sign: no abnormal change reported BW: sign. decreased at days 18 and 21 at 1200 ppm BWG: sign. decreased from D15 to D21 Organ weight: sign. decreased relative ovaries, relative liver, absolute and relative kidney weights at 1200 ppm Food consumption: stat. sign. decreased between days 6-9 and 18-21 at 1200 ppm Parental necropsy: no treatment-related macroscopic modification observed	Anonymous 36, 2017

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Developmental effects: Post-implantation loss: stat. sign. increase in the % of late resorptions and in % of post-implantation loss at 1200 ppm Number of foetuses: stat. sign. decrease in the mean number of foetuses per dam at 1200 ppm Gravid uterus weight: stat. sign. decreased gravid uterus weight at 1200 ppm Pup bw: at 1200 ppm stat. sign. decreased BW at birth, in both sexes Developmental abnormalities (including malformations): stat. sign. increase in the % of pale foetuses per litter, in the % of foetuses with variations per litter, in the % of malformed foetuses per litter, in the % of foetuses with skeletal variations/litter	
Disregarded study Reproductive toxicity study in rat Reliability 4 (according to the registration dossier)	/	Maze learning impaired in all treated groups with histidine diet groups more affected than the nitromethane condition	Whitman *et al.*, 1977
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 premating period, during	**Maternal/paternal effects** Mortality: / Clinical signs: no effects observed BW: a trend to decrease was noted in males and was sign. lower at the highest dose at D7 of the premating period Organ weight: in males: sign. lower FBW and sign. higher relative brain and relative testes weights **Developmental effects** Post-implantation loss: 5.43, 7.98, 3.97 and 7.06 % resp. at 0,	Anonymous 37, 2003

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	mating for both sexes and until gestation day 19 for females	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)	

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 Nitroethane

/

Data on Nitromethane

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

Body weights were statistically significantly decreased at days 18 and 21 in females exposed to the highest dose as compared to controls. This can be explained by a statistically significantly decreased gravid uterine weight in dams of the highest dose group (see Table 90).

No abnormal change was reported in clinical signs.

Table 87: 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 88: 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 89: 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 90: Organ weights (g) in females

Dose (ppm)	0	300	600	1200
Terminal BW (D21)	329.28 ± 22.15	337.51 ± 20.77	326.41 ± 22.04	287.24** ± 24.97
Gravid uterus (g)	76.730 ± 13.817	80.029 ± 14.080	72.779 ± 11.464	35.764** ± 21.653
Empty uterus (g)	4.7554 ± 0.8585	4.9136 ± 0.8269	4.6620 ± 0.5930	3.7435 ± 0.5496
Ovaries (absolute) (g)	0.1186 ±0.0129	0.1283 ± 0.0117	0.1223 ± 0.0140	0.1202 ± 0.0216
Ovaries (relative) (%)	0.0360 ± 0.0036	0.0381 ± 0.0034	0.0375 ± 0.0037	0.0420** ± 0.0071
Placenta (g)	0.44 ± 0.04	0.46 ± 0.05	0.47 ± 0.02	0.42 ± 0.04
Liver (abs) (g)	10.7228 ± 0.9706	11.3909 ± 0.8206	10.9018 ± 0.9298	11.3716 ± 1.0548
Liver (rel) (%)	3.2572 ± 0.2065	3.3789 ± 0.2048	3.3632 ± 0.3029	3.9670** ± 0.2843
Kidneys (abs) (g)	1.3716 ± 0.1276	1.4724* ± 0.1175	1.4840* ± 0.1179	1.6044** ± 1.1222
Kidneys (rel) (%)	0.4175 ± 0.0384	0.4366 ± 0.0276	0.4576 ± 0.0357	0.5623** ± 0.0631

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

Table 91: Reproductive parameters

Dose (ppm)	0	300	600	1200

N	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 92). 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 93 and Table 94. Hematological parameters were not monitored in dams, nor in foetuses.

Table 92: 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 93: 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 94: Skeletal defects in foetuses

Doses (ppm)	0	300	600	1200
N foetuses examined	105	119	118	69
N litters examined	17	19	20	17
Malformations				
N foetuses with skel. malformations (N litters affected)	2 (2/17)	0 (0/19)	1 (1/20)	10 (5/17)
N foetuses with ribs malformed	1	0	0	0
N foetuses with sternebra malformed (%/litter)	0	0	0	9 (10.5**)
Variations				
N foetuses with variations (% per litter)	103 (97.1)	118 (99.1)	114 (95.8)	69 (100)
N 1-4 unossified digits (% per litter)	23 (21.0)	23 (20.1)	25 (20.5)	49 (65.6**)
N incomplete ossification pubis (%/litter)	0	0	0	6 (14.2*)

Wavy ribs	1 (1.0)	3 (2.6)	18 (14.7*)	34 (47.3**)
Incomplete ossification Metatarsals (hindlimbs)	26 (23.1)	20 (17.0)	44 (36.8)	55 (74.9**)

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

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

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

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

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

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

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

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

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

Data on 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 histpathological examination revealed nasal tissue modifications (see chapter 10.10.2 for further information).

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

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-implanatation loss or not. The DS also notes that an even greater percentage in post-implantation loss was observed at 25 ppm, however the mean litter size in the lowest dose group is still similar to the control and mid-dose groups. 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 95). 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 96). Variations and malformations were not examined in the study as well as the physical landmarks.

Table 95: 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 96: Pup body weight data (in g)

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

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 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)." Category 1A: Known human reproductive toxicant The classification of a substance in this Category 1A is largely based on evidence from humans. Category 1B: Presumed human reproductive toxicant The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary nonspecific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.	"Suspected human reproductive toxicant Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be the more appropriate classification. Such effect shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be secondary non-specific consequence of the other toxic effects."

Table 97: 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	Significantly reduced	/	Foetal bw: 4.96, 5.10, 4.98 and	Significant increase incidence of

	2.2, 2.1, 3.9 and 53.8** %	11.9, 11.9, 11.2 and 5.7**		3.93** g in males and 4.80, 4.91, 4.76 and 3.65** g in females	pale foetus at the highest dose (76.5 %/litter) + Sternebra malformed, wavy ribs, incomplete ossification of metatarsal, incomplete ossification of pubis
Nitroethane					
No study available					
1-Nitropropane					
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonyous 37, 2003)	5.43, 7.98, 3.97 and 7.06 %	14.0, 14.3, 15.1 and 11.9 Reduced at the highest dose Not dose related Outside range HCD (13.3 – 15.6)	98.8, 98.8, 100 and 99.2 %	At D 1: 6.7, 6.9, 6.6 and 7.3* g in males and 6.3, 6.5, 6.2 and 6.9* g in females At D 4: 9.2, 9.7, 9.2 and 10.4* g in males and 8.8, 9.2, 8.6 and 9.7* g in females	Not reported

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

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

In a prenatal developmental toxicity study, performed with nitromethane (Anonymous 36, 2017), developmental effects were described. A significant increase was reported in the percentages of late resorptions and post-implantation loss at the highest dose (with 2.2 and 53.8 % post-implantation loss at 0 and 1200 ppm, respectively). Furthermore, a significant decrease was noted in the mean number of foetuses per dam (11.9 and 5.7 at 0 and 1200 ppm, respectively) as well as in foetuses body weights (in average 4.8 and 4.96 g at 0 ppm; and 3.65 and 3.93 g at 1200 ppm, in males and females, respectively). Finally, a significant increase in the number of pale foetuses (0 and 76.5 % per litter, at 0 and 1200 ppm, respectively), in the number of foetuses

with malformations 1.2 and 8.4 % foetuses with malformations, at 0 and 1200 ppm, respectively; the number of litters affected was 2 and 5 out of 17, at 0 and 1200 ppm, respectively) or variations (0.5 and 76.52 % at 0 and 1200 ppm, respectively) and with skeletal malformations (2.2 and 16.4 %, at 0 and 1200 ppm, respectively) were observed. Pale foetuses was an observation consistent with haematological effects seen on the rat after exposure to nitromethane (increased methemoglobinemia, anemia) in the 13-week repeated dose inhalation toxicity study (NTP, 1997; Lewis *et al*., 1977; refer also to chapter 10.12). All these developmental effects appeared at the highest dose only (1200 ppm, equivalent to 2.99 mg/L) in the absence of dose-relationship or severe maternal toxicity. Indeed, no mortality occurred in the dams during the study and no clinical signs are reported. BW, BWG and food consumption were significantly reduced. Food consumption was only significantly reduced during the periods GD 6-9 and GD 18-21, during the reste of the period, it was only slightly reduced. Regarding the reduce BW and BWG, these modifications were expected since the number of foetuses per dams was significantly decreased at the high dose, in comparison with the controls.

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

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

10.10.7 Adverse effects on or via lactation

Table 98: 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
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Rat (SD) 12/sex/dose OECD TG 422 GLP Reliability 1 (according to the registration dossier)	**1-nitropropane** Purity: 99.69 % Inhalation (vapours) Doses: 0, 25, 50 and 100 ppm (corresp. to approx. 0, 0.092, 0.184 and 0.369 mg/L) Duration of exposure: 14 d of premating period, during mating for both sexes and until gestation day 19 for females	**Maternal effects** Mortality: / Clinical signs: no effects observed BW: a trend to decrease was noted in males and was sign. lower at the highest at D7 of the premating period **Pups** Pup BW: sign. higher at 100 ppm in both sexes at lactation day 1 and 4 (within HCD range)	Anonymous 37, 2003

No human data or other relevant studies available

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

In a combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous 37, 2003), groups of 12 male and 12 female SD rats were exposed by inhalation (vapours) to 1-nitropropane (purity: 99.69 %) at a concentration of 0, 25, 50 or 100 ppm (approximatively equivalent to 0, 0.092, 0.184

and 0.369 mg/L, respectively). Females were exposed 14 d prior mating, during mating (2 w) and until the gestation day 19, whereas males were exposed 14 d prior mating and during mating (for a minimum exposure of 28 d). Each female was placed with one male from the same dose level.

The survival index was unaffected (see Table 99). At the highest dose, a significant higher pup body weight was noted in both sexes at D1 and D4 (see Table 100).

Table 99: 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 100: Mean pups body weight (in g)

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

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

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

10.10.9 Comparison with the CLP criteria

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

There is not enough data to conclude on this endpoint 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. Cat. 1B, H360D (May damage 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 101: 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
NITROETHANE			
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 Reliability 2 (according to the registration dossier) Deviation: food consumption not assessed	**Nitroethane** Purity: > 97 % Impurities: Nitromethane < 1 %; 2-Nitropropane < 1.5 % Inhalation: vapours Doses: 0, 100, 350 and 1000 ppm (equivalent to 0, 0.3, 1.0 and 3.0 mg/L, resp.) Duration of exposure: 5/sex/dose for 30 d; 10/sex/dose for 92 d No recovery period, necropsy at the end of exposure period	At 1000 ppm (3 mg/L): Decreased body weight gain Increased MetHb levels with cyanosis, Increased reticulocytes and Heinz bodies in peripheral blood Associated splenic congestion and extramedullary hematopoiesis Degenerative and inflammatory changes in the olfactory nasal epithelium, hepatocellular vacuolization, decreased cytoplasmic granularity of renal cortical tubular epithelium and ductal epithelial cells of the salivary glands At 350 ppm (1 mg/L): Less severe changes in MetHb, spleen, nasal turbinates and salivary glands. At 100 ppm (0.3 mg/L): Minimal changes in MetHb, spleen and salivary glands **LOAEC: 100 ppm**	Anonymous 26, 1982

13-week repeated dose inhalation toxicity study Mice (B6C3F1) 5/sex/dose OECD TG 413 Deviations: yes GLP: Study was initiated prior to GLP and completed with GLP Reliability 1 (according to the registration dossier)	**Nitroethane** Purity: > 97 % Impurities: Nitromethane < 1 %; 2-Nitropropane < 1.5 % Inhalation: vapours Doses: 0, 100, 350 and 1000 ppm (equivalent to 0, 0.3, 1.0 and 3.0 mg/L, resp.) Duration of exposure: 93 d No recovery period, necropsy at the end of exposure period	<u>At 1000 ppm (3 mg/L)</u>: Increased MetHb concentration including the increased presence of reticulocytes and Heinz bodies Moderate degeneration of the olfactory mucosa ± inflammation including moderate glandular hyperplasia Slight increase in cytoplasmic homogeneity of the liver Transient salivary gland alterations of decreased cytoplasmic granularity and decreased eosinophilic staining Presence of multinucleated spermatids in testes <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 **<u>LOAEC: 100 ppm</u>**	Anonymous 26, 1982

Range-finding study for 13-week repeated dose inhalation toxicity study Rat (Fischer 344) 5/sex/dose GLP: Study was initiated prior to GLP and completed with GLP.	**Nitroethane** Purity: unknown Inhalation: vapours Doses: 0, 350, 1000, 2000 or 4000 ppm (equivalent to 0, 1.0, 3.0, 6.0 or 12 mg/L, resp.) Exposure period: 4 d	Please refer to chapter 10.3 (Inhalation acute toxicity study, 4-day study in rats) All animals died at the highest dose: probable cause: hypoxia secondary to methemoglobinemia Specific toxicity from 350 ppm: - cyanosis, a manifestation of the MetHb effect determined in the 13-week study - hyperemia of the nasal turbinates **<u>LOAEC: 350 ppm</u>**	Anonymous 26, 1982
Chronic inhalation toxicity study 2 years Similar to OECD TG 453 GLP compliant: nt specified Rat (Long-Evans) 40/sex/group (control & 100 ppm) 41 males & 39 females (200 ppm) Reliability 2 (according to the registration dossier) Major deviations: - only 2 doses tested - 40 animals / group - some tissues were not examined microscopically (parathyroid, caecum, rectum, bone marrow,…)	**Nitroethane** Purity: 97.92 % Impurities: nitromethane 0.01 % and 2-nitropropane 2.07 % Inhalation 7 h/d, 5 d/w Conc.: 0, 100, 200 ppm (corresp. approx. to 0, 0.31 and 0.61 mg/L, resp.)	*Mortality:* no treatment-related effect *BW*: sign. ↓ at 100 ppm in males and at 200 ppm in females *Clinical chemistry:* slight but sign. ↑ of total protein and BUN in females exposed to 200 ppm *Hematology*: No effects observed. MetHb level not reported. *Organ weights (brain, liver, kidneys, lungs, heart):* no treatment-related effect *Histopathology*: no effect *Neoplastic effects:* No treatment-related increase of tumours In all animals (controls and treated groups), high incidence of benign tumours (adenoma of the pituitary gland) Very rare malign tumours, not treatment-related No HCD available	Anonymous 35, 1986

NITROMETHANE			
16-day repeated dose toxicity study Rat (F344) 5/sex/dose Non-GLP No guideline Not available in the registration dossier, only 90 days study available in the registration dossier but 16 days documented in the same report (NTP, 1997)	**Nitromethane** Purity: > 98 % Inhalation (vapours) Doses: 0, 94, 188, 375, 750 and 1500 ppm (equivalent to 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L resp.). Duration: 16 days, 6 h/d for 5 d/w	1500 ppm (3.750 mg/L) Sign. decreased BWG in males compared to controls *Nervous system*: Sciatic nerve degeneration in 5/5 males and 5/5 females *Respiratory tract*: Degeneration of the olf. epith. in 5/5 males and 5/5 females 750 ppm (1.880 mg/L) *Nervous system*: Sciatic nerve degeneration in 5/5 males and 5/5 females *Respiratory tract*: Degeneration of the olf. epith. in 5/5 males and 5/5 females 375 ppm (0.938 mg/L) *Nervous system*: Sciatic nerve degeneration in 5/5 males and 4/5 females *Respiratory tract*: Degeneration of the olf. epith. in 5/5 males and 5/5 females 188 ppm (0.47 mg/L) and lower No treatment-related effect in males and females **LOAEC: 375 ppm**	NTP, 1997

16-day repeated dose toxicity study Mouse (B6C3F) 10/sex/dose Non-GLP No guidelineNot available in the registration dossier, only 90 days study available in the registration dossier but 16 days documented in the same report (NTP, 1997)	**Nitromethane** Purity: > 98 % Inhalation (vapours) Doses: 0, 94, 188, 375, 750 and 1500 ppm (equivalent to 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L resp.). Duration: 16 days, 6 h/d for 5 d/w	1500 ppm (3.750 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; Increased absolute and relative liver weight in males and females 750 ppm (1.880 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; Increased absolute and relative liver weight in males and females 375 ppm (0.938 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; Increased absolute and relative liver weight in females. Increased relative liver weight in males. 188 ppm (0.47 mg/L) Increased absolute and relative liver weight in females 94 ppm (0.235 mg/L) Increased absolute and relative liver weight in females **LOAEC: 375 ppm**	NTP, 1997

13-week repeated dose toxicity study Rat (Fischer 344) 10/sex/dose Similar to OECD TG 413 GLP-compliance not specified Reliability 1 (according to the registration dossier)	**Nitromethane** Purity: > 98 % Inhalation (vapours) Doses: 0, 94, 188, 375, 750 and 1500 ppm (equivalent to 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L resp.). Duration: 13 weeks, 6 h/d for 5 d/w	<u>1500 ppm (3.750 mg/L)</u> Decreased FBW (-12%) and BWG (-19%) in males compared to controls *Nervous system*: Hindlimbs paralysis in 10/10 males and 10/10 females from day 21; Decreased hindlimb (males and females) and forelimb grip strength (only males); Sciatic nerve and spinal cord degeneration in 10/10 males and 10/10 females Startle response amplitude decreased in males and females *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females + hyaline droplets in 8/10 males and 10/10 females Bone marrow hyperplasia in 10/10 males and 10/10 females Goblet cells hyperplasia in 10/10 males and 10/10 females Sign. decrease in T3, thyroxine and free thyroxine in both sexes at day 23 Sign. increase in erythrocytes and MetHb levels at week 13 Sign. decrease in the weight of left cauda, epididymis and testis <u>750 ppm (1.880 mg/L)</u> *Nervous system*: Sciatic nerve and spinal cord degeneration in 10/10 males and 10/10 females Startle response amplitude decreased in males and females *Respiratory tract*: Degeneration of the olf. epith. 10/10 males and 10/10 females; hyaline droplets in 4/10 females Bone marrow hyperplasia in 9/10 males and 7/10 females Significant increase in erythrocytes and MetHb levels at week 13 <u>375 ppm (0.938 mg/L)</u> *Nervous system*: Sciatic nerve (5/10 males and 8/10 females) and spinal cord (9/10 males) degeneration Startle response amplitude decreased in males	NTP, 1997

		Respiratory tract: Degeneration of the olf. epith. in 9/10 males and 10/10 females Bone marrow hyperplasia in 6/10 females Sign. increase in erythrocytes and MetHb levels at week 13 <u>188 ppm (0.47 mg/L) and lower</u> Sign. increase in erythrocytes and MetHb levels at week 13 **LOAEC (systemic, male/female): 188 ppm (0.470 mg/L)** based on disturbance of hematological parameters **NOAEC (systemic, male/female): 94 ppm (0.235 mg/L)** **LOAEC (local, male/female): 375 ppm (0.938 mg/L)** for the upper respiratory tract **NOAEC (local, male/female): 188 ppm (0.470 mg/L)**	

13-week repeated dose toxicity study Mouse (B6C3F1) 10/sex/dose Similar to OECD TG 413 GLP-compliance not specified Reliability 1 (according to the registration dossier)	**Nitromethane** Purity: > 98% Inhalation (vapours) Doses: 0, 94, 188, 375, 750 and 1500 ppm (equivalent to 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L resp.). Duration: 13 weeks, 6 h/d for 5 d/w	1500 ppm (3.750 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; hyaline droplets in 10/10 males and 10/10 females *Spleen*: extramedullary hematopoiesis in 10/10 males and 9/10 females Increased absolute and relative kidney weight in females. Increased absolute and relative liver weight in males Sign. decrease in sperm motility (82.41 % v.s. 93.50 in controls) 750 ppm (1.880 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; hyaline droplets in 10/10 males and 10/10 females Increased absolute kidney weight in males and females. Increased absolute and relative liver weight in males Sign. decrease in sperm motility (86.47 % v.s. 93.50 in controls) 375 ppm (0.938 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 10/10 males and 10/10 females; hyaline droplets in 10/10 males and 10/10 females Increased absolute kidney weight in males. Increased absolute and relative kidney weight in females. Increased relative liver weight in males Sign. decrease in sperm motility (85.09 % v.s. 93.50 in controls) 188 ppm (0.47 mg/L) *Respiratory tract*: Degeneration of the olf. epith. in 7/10 females; hyaline droplets in 1/10 males and 9/10 females Increased absolute kidney weight in males and females. 94 ppm (0.235 mg/L) No treatment-related effect in males and females	NTP, 1997

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

Sub-chronic 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)	**Nitromethane** Purity: 96.5% Inhalation (vapours) Doses: 100 and 750 ppm (equivalent to 0.25 and 1.875 mg/L, resp.) Duration: 13 weeks and up to 24 weeks, 7 h/d for 5 d/w	<u>750 ppm (1.875 mg/L)</u> Reduced T4 levels at all time points Reduced Hb levels at 1-month Increased OCT levels at 1 and 3-month <u>100 ppm (0.25 mg/L)</u> Reduced T4 levels at all time points Reduced Hb levels at 1-month Increased OCT levels at 1 and 3-month Increased thyroid gland weights after 6-months of exposure, dose not specified. *Lung* : at 1-month, interstitial edema, moderate to moderately severe focal hemorrhage and sometimes necrosis in the area of hemorrhage. Frank edema in some animals. Dose not specified. **LOAEC (male): 98 ppm (0.25 mg/L)** based on reduced T4 levels throughout the study **No NOEC**	Lewis *et al.,* 1977

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

2-year repeated dose inhalation study Equivalent or similar to OECD TG 451 GLP-compliant 2 years Mice (B6C3F1) 50/sex/group Reliability 1 (according to the registration dossier) *Guidance value range for warranting classification as cat. 1: ≤ 0.025 mg/L/d and as cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d*	**Nitromethane** Purity: > 99 % Impurities: 0.25 % nitroethane, 0.03 % 2-nitropropane inhalation 6 h/d, 5 d/week 0, 188, 375, 750 ppm (approx. equivalent to 0, 0.47, 0.94 and 1.87 mg/L, resp.)	*Mortality:* 38, 28, 40 and 42 % of M and 50, 44, 48 and 28 % of F exposed to 0, 188, 375 and 750 ppm, resp. *Clinical sign:* in the eyes, swelling and exophthalmos coincident with harderian gland tumours, in both sexes *BWG:* no effects in males, slightly increased BW in females during the study but similar to controls at study termination *Organ weights:* no data *Histopathology:* - sign. increased 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 respiratory epith. hyaline degeneration in all treated groups in females and at the middle and high doses in males. *Neoplastic effects* **Harderian gland**: Male and female: Adenoma (%): M: 9/49 (18), 10/50 (20), 19/50 (38), 32/49 (65) F: 5/49 (10), 7/49 (14), 16/50 (32), 19/50 (38) Carcinoma (%): M: 1/49 (2), 1/50 (2), 6/50 (12), 5/49 (10) F: 1/49 (2), 2/49 (4), 4/50 (8), 3/50 (6) Adenoma or carcinoma (%): M: 10/49 (20), 11/50 (22), 25/50 (50), 37/50 (74) F: 6/49 (12), 9/49 (18), 20/50 (40), 21/50 (42) **Liver**: Female (%): Hepatocellular adenoma:	NTP, 1997

		F: 14/50 (28), 25/49 (51), 17/49 (35), 35/50 (70) Hepatocellular carcinoma: F: 10/50 (20), 14/49 (29), 8/49 (16), 12/50 (24) Hepatocellular adenoma or carcinoma: F: 19/50 (48), 34/49 (69), 22/49 (45), 40/50 (80) No increase in liver tumours was observed in Males. **Lung:** Male and female (%): Alveolar / bronchiolar adenoma M: 11/50 (22), 10/50 (20), 9/50 (18), 12/50 (24) F: 3/50 (6), 3/50 (6), 2/49 (4), 9/50 (18) Alveolar / bronchiolar carcinoma M: 2/50 (4), 3/50 (6), 3/50 (6), 11/50 (22) F: 0/50 (0), 3/50 (6), 5/49 (10), 3/50 (6) Alveolar / bronchiolar adenoma or carcinoma M: 13/50 (26), 13/50 (26), 12/50 (24), 20/50 (40) F: 3/50 (6), 6/50 (12), 6/49 (12), 12/50 (24)	

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

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

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

Table 102: Summary table of human and other studies relevant for STOT RE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Case study report	**Nitroethane** Purity: 100 % Oral exposure Quantity < 1 ounce (less than 30 mL)	Human 1 boy 20-month old	Cyanosis Methemoglobinemia level: increased to 39 % Full recovery after intravenous methylene blue injection	Hornfeldt and Rabe, 1994
Case study report	**Nitroethane** Purity: 100 % Oral exposure Quantity: max. 90 mL	Human 1 girl 13-month old	Cyanosis, tachypnea, lethargy, emesis 7 h after ingestion. Methemoglobinemia up to 53 % 23 h after ingestion.	Osterhoudt *et al.*, 1995
Disregarded study **Neurotoxicity study** No guideline Reliability 4 (according to the registration dossier) GLP: not specified Rat SD Male/female 4-5 animals in each group	**Nitroethane** Purity: unknown 275 mg/kg Oral: gavage Two hours after a single acute oral dose of nitroethane, the profile of several neurochemicals in the brain was examined.	Disregarded study: origin of the effects are not described (direct/indirect effect due to hypoxia)	Increased levels of MHPG and 5HIAA in treated groups but as it was previously shown that nitroethane administered repeatedly could cause elevated methemoglobinenia, it is complicated to conclude if it is due to a direct effect of nitroethane or indirect via a decrease in oxygen levels in the brain	Kanada *et al.*, 1994

Hepatotoxicity No guideline GLP: not specified Reliability 2 (according to the registration dossier) BALB/c mice Male/female: 19-25 g 3-5/sex/dose	**Nitroethane** Purity: unknown 4.5, 6.7 or 9.0 mmol/kg IP	Reporting deficiencies (doses not clearly stated for example)	No sign. increase in SDH, ALT or AST activity. No significant abnormalities in livers of mice exposed to 9 mmol/kg	Dayal R *et al.,* 1989

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

Data on 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/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).

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

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

No death occurred during the experiment.

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

Table 103: 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*
326 ± 14	322 ± 20	316 ± 11	293 ± 8*	62	90	194 ± 10	190 ± 10	184 ± 7*	176 ± 7*

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

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

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

- Female rats of the 100, 350 and 1000 ppm exposure groups had an unkept appearance which was an expression of their general weakened condition, secondary to the toxicity of the test material.

Two other clinical findings, swelling in the salivary gland region and increased amounts of porphyrin pigments around the nares, were observed in some rats of the 100, 350 or 1000 ppm group. These observations were consistent with a mild transient viral infection (sialodacryoadenitis) which commonly occured in this laboratory and were not judged to be treatment-related.

Prior to interim kill (20th exposure day, D29 of the experiment), methemoglobin was dosed in blood, 15 hours after the last exposure (Part A of Table 104). 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 104).

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 104).

At terminal kill, a time-sequenced analyse (Part D of Table 104) 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 104: Methemoglobinemia

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

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

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

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

Table 105: Haematological parameters

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

PCV= packed cells volume (%); RBC= Red blood cells ($x10^6/mm^3$); Hb= Hemoglobin (g/100ml); WBC= White blood cells ($x10^3/mm^3$); Reticulocytes (%); Heinz bodies (%); *p<0.05

Histological assessment is described in Table 106. 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 106: Histopathological assessment

	Males				**Females**			
Dose levels (ppm)	0	100	350	1000	0	100	350	1000
At interim sacrifice (D30)								
N	5	5	5	5	5	5	5	5
With N tissues examined	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Liver: slight mononuclear cells aggregates	1	2	1	1	1	1	1	1
Slight mononucl. aggreg. In the portal area	0	1	1	0	0	0	0	0
Slight focal extramedullary hematopoiesis	0	0	1	0	0	0	0	0
Focal granulomatous inflammation	0	0	0	1	0	0	0	0
Focal necrosis	0	0	0	1	0	0	0	0
Slight diffuse vacuolization	0	0	0	3	5	4	5	5
hernia	0	0	0	0	1	0	1	0
Heart: slight focal inflam. myocardium	0	3	0	0	0	0	0	0
Slight multifocal inflam. myocardium	0	0	0	0	0	1	0	0

Slight Focal subacute inflam.	1	0	0	0	0	0	0	0
Slight Focal subacute myocardial inflam.	1	0	0	1	0	0	0	0
Spleen: congestion	0	0	5	5	5	5	5	0
Extramedullary hematopoiesis	0	0	2	5	0	0	0	3
Kidney: decreased tubules cytop. granularity	0	0	0	2	0	0	0	0
Slight focal cortical basophilia	0	0	0	0	1	0	1	0
Slight subacute focal interstitium: inflam.	0	0	0	0	0	1	0	0
Slight focal mineralization CJ	0	0	0	0	0	1	2	0
Slight multifoc. Mineralization CJ	0	0	0	0	2	2	0	0
Lungs: slight multifoc. Mononucl. Aggreg: peribroncholar area	5	5	5	5	5	5	5	5
Slight focal mononucl. Aggreg. Subpleural area	0	1	1	0	0	1	0	1
Slight multifoc mononucl. Aggreg. Subpleural area	0	0	0	1	0	0	1	0
Slight focal mononucl. aggreg. Blood vessels	0	1	1	0	0	0	0	0
Slight Focal subacute inflam. subpleural area	0	0	1	0	0	0	0	0
Nasal turbinates: slight focal mononucl. Aggregates submucosa area	0	0	0	1	3	0	0	0
Slight multifocal mononucl. Aggreg. Submucosa area	5	5	5	4	2	5	4	4
Slight focal degeneration, olfactory epith.	0	0	0	0	0	0	3	0
Slight multifoc. Degen, olfactory epith.	0	0	2	0	0	0	0	0
Slight diffuse degeneration, olf. Epith.	0	0	3	5	0	0	0	5
Slight chronic active inflam. Olf. epithelium	0	0	5	5	0	1	1	5
With N tissues examined	**5**	**0**	**0**	**5**	**5**	**0**	**0**	**5**
Adrenal: slight extramed. hemotopoiesis	0	-	-	0	1	-	-	0
Stomach: diffuse nongland. Submuc. edema	0	-	-	0	0	-	-	1
Diffuse submucosa edema	0	-	-	0	0	-	-	1
Cecum: parasites: nematode	1	-	-	0	0	-	-	0
Large intestine: parasites: nematode	0	-	-	1	0	-	-	1
Cervical lymph nodes: erythrophagocytosis	0	-	-	0	0	-	-	1
Salivary gland: slight acini vacuolization	5	-	-	5	0	-	-	0
Mammary gland: N tissues examined	4	0	0	4	5	-	-	5
Slight acini hyperplasia	4	-	-	4	0	-	-	0
Slight ducts hyperplasia	0	-	-	0	5	-	-	5
At terminal kill								
N animals	5	5	5	5	5	5	5	5
With N tissues examined	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Liver: slight focal aggregates of mononuclear cells	0	0	0	0	0	0	0	1
Diaphragmatic hernia causing altered architecture	0	0	0	0	2	0	0	0
Very slight mutifoc extramed. Hematopoiesis	2	0	0	1	0	0	0	0
Slight multifocal extramed. Hematopoiesis	0	0	0	0	0	0	0	1
Subcapsular fibrosis	0	0	0	0	0	0	0	1
Focal subcapsular fibrosis	0	0	0	0	1	0	0	0
Subcapsular hematogenous pigment	0	0	0	0	0	0	0	1

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

Very slight ductal decreased cytop. granularity	0	5	0	0	0	5	0	0
Slight decrease in ductal cytop. granularity	0	0	5	5	0	0	5	5
Very slight decreased ductal eosinophilia	0	5	0	0	0	5	0	0
Slight decreased ductal eosinophilia	0	0	5	5	0	0	5	5
Acini vacuolization	0	0	0	0	0	0	0	3
Trachea: N tissues examined	5	5	5	5	5	5	5	5
Slight focal mononucl aggreg. Submucosa	0	2	2	0	2	1	0	0
Mammary gland: N tissues examined	2	3	1	1	4	3	5	5
Slight acini hyperplasia	1	1	1	1	0	1	0	0
Slight ductal hyperplasia	0	0	0	0	0	0	1	1
Eye: N tissues examined	5	5	4	5	5	5	5	5
Decreased size	0	0	1	0	0	0	0	0
Fibrosis	0	0	1	0	0	0	0	0
Fibrosis, posterior chamber area	0	0	0	1	0	0	0	0
Haemorrhage	0	0	0	0	0	1	0	0
Unilateral haemorrhage	0	0	0	0	0	1	0	0
Unilateral hematogenous pigment	0	0	0	0	0	1	0	0
Osterior chamber hematogenous pigment	0	0	0	1	0	0	0	0
Nasal turbinates: N tissues examined	5	5	5	5	5	5	5	5
Slight multifoc mononucl aggreg, submucosa	5	5	5	5	5	5	5	5
Slight focal degeneration olfactory epith	0	0	1	0	0	0	0	0
Slight diffuse degen. Olf. Epith.	0	0	1	0	0	0	2	0
Moderate diffuse degen. Olf. Epith.	0	0	0	5	0	0	0	5
Moderate multifoc. degen. Respiratory epith.	0	0	0	1	0	0	0	0
Slight acute inflammation Resp. epith	0	0	1	0	0	0	0	0
Slight multifocal acute infla. Vomeronasal organ	0	1	0	0	0	0	0	0
Slight focal chronic active infla. Olf. epith	0	0	1	0	0	0	0	0
Slight multifocal Chronic Active inflammation Olfactory epithelium	0	0	1	0	0	0	0	0
Slight diffuse chronic active infla. Olf. Epith	0	0	0	4	0	0	2	5
Moderate diffuse chronic active infla. Olf. epith	0	0	0	1	0	0	0	0
Slight diffuse subacute inflammation of respiratory epithelium	0	0	0	1	0	0	0	0
Slight focal metaplasia of resp. epith.	1	0	0	0	0	0	0	0

CJ= corticomedullary junction

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

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

In a sub-chronic repeated dose toxicity study (Anonymous 26, 1982), groups of B6C3F1 mice were exposed to 0, 100, 350 or 1000 ppm (0, 0.3, 1.0 or 3.0 mg/L) of nitroethane for 6 h/d, 5 d/w for a total of 64-65 exposures (over a 93-d period) with an interim sacrifice of rats after 20-21 exposures (over a 29-d period). Parameters monitored were clinical observations, body weights, organ weigths, hematological characteristics including methemoglobin (MetHb) determination, clinical chemistries, gross pathology and histopathology.

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

The results obtained show an increased methemoglobinemia, effects in the salivary glands, liver, olfactory nasal epithelium and multinucleated spermatids in the testes at 1000 ppm. At 350 ppm, methemoglobinemia, effects in the liver, salivary glands and nasal epithelium were seen. At the lowest dose, minimal effects were reported in the nasal epithelium, and transient effects on the epithelium of the salivary glands.

The statistically significant changes found in the PCV, RBC and Hb parameters at the interim and terminal analysis were within the normal variability for the B6C3F1 mouse. Increased reticulocytes and Heinz bodies were detected in the mice of the 350 and 1000 ppm groups at the interim and terminal kills.

Table 107: Haematological parameters

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

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

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

100 and 350 ppm groups and in males exposed to 1000 ppm. The level was however significantly increased at 1000 ppm, in females.

Table 108: Methemoglobinemia

Males					Females			
0	100	350	1000	Dose levels	0	10	350	1000
5	5	5	5	N	5	5	5	5
Immediately after the 64th (last) exposure (D92)								
0.8 ± 0.3	1.2 ± 0.4	6.6* ± 4.3	36.4* ± 3.0	MetHb	1.2 ± 0.7	0.9 ± 0.7	5.8* ± 1.8	20.8* ± 2.0
4h after last exposure								
Not det.	Not det.	Not det.	7.4 ± 2.6	MetHb	Not det.	Not det.	Not det.	10.4 ± 2.9
19h after last exposure								
0.8 ± 0.7	0.8 ± 0.4	1.3 ± 1.0	0.9 ± 0.4	MetHb	1.1 ± 0.3	0.9 ± 0.6	1.3 ± 0.4	2.4* ± 0.8

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

Prior to the interim kill (30 days), no effects were seen on SGPT (serum glutamic-pyruvic transaminase) and calcium blood levels of males and females.

Table 109: 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); *p<0.05

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

Table 110: Clinical biochemistry parameters at terminal kill

Males				**Exposure (ppm)**	Females			
0	100	350	1000		0	100	350	1000
At terminal kill (on mice from which blood was *already punctured the day before* to assess MetHb)								
38±6	36±10	44±12	30±4	**BUN**	29±3	21*±2	25±4	33±5
39±6	46±7	43±7	37±2	**AP**	59±7	58±7	53±15	49±7
8.2±0.6	9.4±0.5	9.6±0.6	8.8±2.1	**P**	8.9±1.1	7.5±0.7	6.9±2.1	8.4±1.0
At terminal kill (on mice *never bled* before)								
34±5	29±2	20*±2	27±6	**BUN**	26±4	21±3	19*±2	20*±3
45±6	36±5	38±4	39±7	**AP**	54±8	60±7	55±6	63±12
10.7±2.0	8.3±0.3	9.3±1.9	9.4±1.0	**P**	8. 2±0.6	7.3±1.2	8.0±0.9	8.4±1.1
10.5±0.6	11.2±0.8	9.9±0.3	10.0±0.2	**Ca**	10.2±0.2	10.0±0.5	9.8±0.2	9.6*±0.1

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

Prior to the interim kill (30 days), no changes were found in absolute liver, kidney, and brain weights in both sex. No changes in absolute heart weights, nor in absolute and relative thymus and testes weights in males were reported as well. In females, heart absolute weights were slightly decreased in all treatment groups (0.13±0.01, 0.11*±0.01, 0.10*±0.01 and 0.10*±0.01 at 0, 100, 350 and 1000 ppm, respectively) while mean relative heart weigths in females were only significantly decreased at the highest dose level (0.50±0.04, 0.45±0.05, 0.45±0.03 and 0.42*±0.04 at 0, 100, 350 and 1000 ppm, respectively). Furthermore, no changes in kidney relative weights were seen in females.

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

Table 111: Organ weights

	Males				**Females**			
Dose levels (ppm)	0	100	350	1000	0	100	350	1000
At interim kill								
N	5	5	3	4	5	5	5	5
Mean BW	27.4±0.9	28.4±2.5	28.3±1.5	27.3±1.7	26.2±1.3	24.0±0.7	23.4±2.7	23.8±1.6
Liver (rel) (%)	6.08±0.26	5.64±0.21	5.20*±0.24	6.06±0.3	5.45±0.21	5.40±0.34	5.44±0.26	6.36*±0.25
Kidney (rel) (%)	2.04±0.13	1.75*±0.11	1.72*±0.2	1.76*±0.11	No changes			
Thymus (abs) (g)	No changes				0.06±0.01	0.04*±0.00	0.03*±0.01	0.02*±0.01
Thymus (rel) (%)	No changes				0.23±0.03	0.18*±0.02	0.14*±0.05	0.10*±0.02
At terminal kill								
Mean BW	34.3±2.0	33.6±2.5	32.4±2.6	32.4±2.5	27.4±1.8	28.1±1.4	27.7±1.4	28.4±1.6
Kidney (rel) (%)	No changes				1.38±0.11	1.47*±0.04	1.49*±0.06	1.42±0.1
Heart (rel) (%)	No changes				0.49±0.06	0.49±0.05	0.42*±0.03	0.41*±0.03
Brain (abs) (g)	No changes				0.46±0.02	0.47±0.02	0.45±0.02	0.43*±0.02
Brain (rel) (%)	No changes				1.69±0.12	1.66±0.08	1.63±0.05	1.53*±0.09
Thymus (abs) (g)	0.04±0.01	0.03±0.01	0.03±0.01	0.02*±0.01	No changes			
Thymus (rel) (%)	0.11±0.03	0.09±0.04	0.08±0.02	0.08*±0.03	No changes			
Testes (abs) (g)	0.22±0.02	0.22±0.02	0.23±0.02	0.23±0.02	N/A			
Testes (rel) (%)	0.64±0.06	0.65±0.05	0.70*±0.05	0.72±0.03	N/A			

N/A: not applicable; rel= relative; abs= absolute

At interim kill, no macroscopic lesions were seen in males and females, except for alopecia in the thoracic area of 1/3 males exposed to 350 ppm.

At terminal kill, no gross findings were reported except for:

- At 100 ppm: severe unilateral decrease in the size of a testicle and epidydimis in 1/10 males, unilateral preputial abscess in 1/10 males, and moderate alopecia on the abdomen and thorax (probably the same animal) on 1/10 females.

- At 350 ppm: a slightly increased spleen in 1/8 males and one focal preputial ulcer was reported in 1/8 males.

- At 1000 ppm, an ovary nodule in 1/10 females

Concerning histopathological findings, prior to the interim kill (30 days), hepatocellular vacuolization consistent with fat changes were noted in females exposed to 1000 ppm.
Slight focal glandular granuloma in the stomach submucosa and slight focal chronic active submucosal inflammation were seen in 1/4 control male, however, it is not mentioned if it was the same animal that was affected. Dermoid cyst in meninges and ectopic thymic tissue was reported in 1/4 control female, however, it is not specified if it was the same animal affected.
At terminal kills (92 days): Slight multifocal mineralization of the myocardium was reported in 1/5 control male. Focal dermoid cysts in spinal cord meninges was seen in 1/5 control female. Multifocal mononuclear cells aggregates were seen in 2/5 control females.

Table 112: Histopathological modifications

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

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

Primary benign teratoma, no metastasis	-	-	-	-	0	0	0	1
Cervix: N tissues examined	-	-	-	-	4	0	0	5
Very slight focal muscularis acute inflam.	-	-	-	-	0	0	0	1
Lacrimal gland: N tissues examined	2	1	2	1	1	0	0	2
Moderate acute inflammation	0	0	0	0	0	0	0	1
Moderate unilateral acute inflammation	1	0	0	1	0	0	0	0
Slight focal unilateral acute inflammation	0	1	1	0	1	0	0	0
Slight multifocal unilateral actue inflammation	0	0	1	0	0	0	0	0
Moderate multifocal unilateral acute inflammation	1	0	0	0	0	0	0	1

C.G.= cytoplasmic granularity; Z.G.= zona glomerula; unilat.= unilateral; bilat.= bilateral

1, 0, 2 and 1 male mice died during the experiment in groups exposed to 0, 100, 350 and 1000 ppm nitroethane, respectively. No macroscopic lesions were reported except, at 350 ppm, thymus atrophy in 1/2 male, decreased abdominal fat in 1/2 male, loss of body condition in 1/2 male, and slight soiled perineum in 1/2 male.

Histopathologic examination in mice dying spontaneously did not show effects except for:

- Slight multifocal submucosa mononuclear aggregates in 1/2 males exposed to 350 ppm
- Moderate degeneration of the olfactory epithelium, without or with inflammation in 2/2 and 1/1 males exposed to 3502 and 1000 ppm, respectively
- Moderate glandular hyperplasia in the olfactory epithelium in 1/2 and 1/1 males exposed to 350 and 1000 ppm, respectively

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 44)

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

Please refer to chapter 10.9.1.

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

Case report

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

In another case study report (Osterhoudt *et al.*, 1995), a 13-month old girl ingested fingernail polish remover first thought to be acetone-based. She weighted 10.2 kg, was healthy and under no medication. She first was brought to the emergency room without any symptom and sent home. Then 7 hours after ingestion, she came back and presented emesis and lethargy. The fingernail product was identified as 100 % nitroethane and maximum 90 mL was missing from the bottle. Cyanosis and tachypnea were observed. Oxygen (80 % supplement) was given but the girl remained in a cyanotic state. No cardiac symptom were reported; nor abdominal abnormalities. Methemoglobinemia was confirmed with blood analysis (Table 113**Error! Reference source not found.**). A rebound in methemoglobin increased its level up to 53 % 23 hours after ingestion.

Table 113: 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 Nitromethane

Oral exposure

In a sub-chronic repeated dose toxicity study (Weatherby *et al.*, 1955), groups of 10 male and 10 female albino rats were orally exposed to nitromethane in drinking water for 15 weeks. Doses chosen were 0, 0.1, 0.25, 0.5, 1 and 2 % but doses starting from 0.5 % were not supported by the animals and therefore were abandoned after a week. Only the control and 0.1 and 0.25 % groups were kept, corresponding to an average daily intake of 150 and 285 mg/kg bw/day nitromethane, respectively. Moreover, 4 and 3 animals out of 10 died in groups exposed to 0.1 and 0.25 %, respectively.
In surviving animals, necropsy was performed and tissues examined. A the end of exposure period, gross and microscopic changes were assessed in the heart, lungs, liver, spleen, kidney, testes, adrenal gland and small intestine.
Decreased body weight was noted in surviving animals at 0.1 and 0.25 % (no more information available). Histopathological findings indicated larger hepatic cells with a prominent nucleus in 2/6 surviving animals in the 0.1% group exposed to 0.1 % nitromethane. In the 0.25% group, 2/7 surviving animals had more prominent Malpighian corpuscles compared to normal spleen. In 6/7 animals, the liver cells cytoplasms were less stained and more granular compared to control group, and more lymphocytes were noted in the periportal zone.
All animals in the control group survived, 1/10 rats had large hepatic cells with prominent nuclei.

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

Inhalation

In a 16-day repeated dose toxicity study (NTP, 1997), groups of 5 male and 5 female rats were daily exposed to 0, 94, 188, 375, 750 or 1500 ppm (equivalent to 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L, respectively) nitromethane by inhalation for 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 114: 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 ≤ C ≤ 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 effets at a higher dose. Calculated doses for a shorter study via the Haber's rule may lead to unclear relevance of the effects. However, the DS notes that the early onset of neurological and respiratory effects can be supportive of a classification for STOT RE (nervous system and respiratory tract).

In another 16-day repeated-dose 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 ≤ C ≤ 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 115: 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 116: 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 117: Histopathological findings

Exposure level (ppm)		**0**	**94**	**188**	**375**	**750**	**1500**
♂	**N**	10	10	10	10	10	10
	Bone marrow hyperplasia	0	0	0	0	9**	10**
	Degeneration olf. epithelium	0	No animal tested	0	9**	10**	10**

	Hyaline droplets, olf. epithelium	0	No animal tested	0	0	1	8**
	Hyperplasia Goblet cells	0	No animal tested	0	0	1	10**
	Sciatic nerve degeneration	0	No animal tested	0	5*	10**	10**
	Spinal cord degeneration	0	No animal tested	0	9**	10**	10**
♀	**N**	10	10	10	10	10	10
	Bone marrow hyperplasia	0	0	1	6**	7**	10**
	Degeneration olf. epithelium	0	0	1	10**	10**	10**
	Hyaline droplets, olf. epithelium	0	0	0	0	4*	10**
	Hyperplasia Goblet cells	0	0	0	0	2	10**
	Sciatic nerve degeneration	0	No animal tested	0	8**	10**	10**
	Spinal cord degeneration	0	No animal tested	0	2	10**	10**

The LOAEC (systemic, male/female) was determined as 188 ppm, the NOAEC (systemic, male/female) was 94 ppm based on disturbance of hematological parameters at 188 ppm, the LOAEC (local, male/female) was 375 ppm for the upper respiratory tract, and the NOAEC (local, male/female) was 188 ppm.

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

In a 13-week repeated dose toxicity study (NTP, 1997), groups of 10 male and 10 female B6C3F1 mice were exposed by inhalation to 0, 94, 188, 375, 750 and 1500 ppm (equivalent to 0, 0.235, 0.470, 0.938, 1.880 and 3.750 mg/L, respectively) nitromethane during 6-h per day, for 5 d/week during 13 weeks. Clinical signs and body weight were observed weekly. Additional groups of 5 mice per sex were included before the starting of the study for parasite and clinical pathology assessment and the kidneys of 5 mice/sex were removed and evaluated. At the termination of the study, a serologic examination was performed on 5 mice/sex and all mice were also necropsied for clinical pathology evaluation.

No effects were reported on body weight and body weight changes at any dose. In males, a significant increase of the relative liver weight starting at 375 ppm and of absolute right kidney weights (except at 1500 ppm), in comparison with the controls was observed. In females, a significant increase of the relative and absolute weights of kidneys at 750 and 1500 ppm, in comparison with the controls, was reported.

Olfactory epithelial degeneration and respiratory epithelial hyaline droplets were observed mixroscopically 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 118: Histopathological findings

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

The LOAEC (systemic, male/female) was determined as 188 ppm based on the modification of some organ weights, the NOAEC (systemic, male/female) was 94 ppm based on the effects seen at 188 ppm on organ weights, the LOAEC (local, male/female) was 375 ppm for the upper respiratory tract, and the NOAEC (local, male/female) was 188 ppm.

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

In another sub-chronic inhalation repeated dose toxicity study (Lewis *et al.*, 1977), male rats were exposed by inhalation to 100 and 750 ppm nitromethane (equivalent to 0.25 and 1.875 mg/L, respectively) for 13 weeks, and up to 24 weeks. Body weights and body weight gains were followed up regularly. 10 Animals from each dose group were sacrificed by phenobarbital overdose and exsanguinated at different time points where blood hematology and biochemistry as well as several tissue examinations (lungs, liver, kidney, trachea, brain, thyroid) were analysed (after 2 d, 10 d, 1 month, 3 months, 6 months).

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

Hematocrit level was significantly decreased in rats exposed to 750 ppm at all time points, except at day 2. When exposed to 100 ppm, the hematocrit level was only decreased at the day 10 time point. Hemoglobin level was significantly decreased at all time points when rats were exposed to 750 ppm, however, in rats exposed to 100 ppm, the decrease was only seen at the day 10 time point. Red blood cells counts increased in the group exposed to 750 ppm at the 2-day time point, but they were decreased at the day10, 1-month and 3-month time points. The difference with the control group was not significant only at the day10 time point. When rats were exposed to 100 ppm, the red blood cells counts were only increased at the 10-day time point, compared to controls. There were no treatment-related effects in methemoglobin and prothrombin concentrations.

Table 119: Hematological parameters

Parameters	Dose level (ppm)	Day 2	Day 10	Month 1	Month 3	Month 6
Ht	0	39 ± 0.5	41 ± 0.5	44 ± 0.3	44 ± 0.7	43 ± 0.5
	750	40 ± 0.9	39 ± 0.9*	42 ± 0.4***	41 ± 0.3***	40 ± 0.8**
Hb	0	10.8 ± 0.22	13.9 ± 0.21	14.6 ± 0.13	14.8 ± 0.23	14.0 ± 0.23
	750	11.1 ± 0.21	12.9 ± 0.25***	13.7 ± 0.17***	13.0 ± 0.22***	12.3 ± 0.22***
RBC	0	5.61 ± 0.111	6.31 ± 0.97	6.89 ± 0.112	6.47 ± 0.123	7.79 ± 0.127
	750	6.03 ± 0.123*	5.89 ± 0.116*	6.68 ± 0.064	6.05 ± 0.068**	7.71 ± 0.128
MetHb	0	0 ± 0.1	0.08 ± 0.007	0.06 ± 0.008	0.08 ± 0.022	0.01 ± 0.002
	750	0 ± 0.1	0.08 ± 0.006	0.10 ± 0.029	0.08 ± 0.011	0.07 ± 0.058
PT time	0	15.1 ± 1.17	14.2 ± 0.12	15.1 ± 0.49	15.8 ± 0.31	14.6 ± 0.28
	750	16.8 ± 1.58	13.7 ± 0.20*	14.6 ± 0.25	15.6 ± 0.26	14.8 ± 0.34

With * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; results at 100 ppm are not available

Ornithine carbamyl transferase (OCT) levels were increased at the 10-day time point in rats exposed to 750 ppm. T4 concentrations were reduced at the 2-day time point in rats.

After a 2-day, 10-day and 1-month exposure to nitromethane, no macroscopic effects were seen at both doses. At the 3-month time point, “whitish or greyish” focal areas in the lung were seen in both exposure groups. At the 6-month time point, a significant increase in the incidence of white focal areas scattered on all lungs lobes of the exposed and control group was reported as well as a decrease in the number of focal hemorrhages on the lungs. Pale kidneys were also reported in control and treated groups. Concerning organ weights, the lung weights tended to decrease at all time points. At the 6-month time point, the thyroid gland weights were increased in the group exposed to 750 ppm, in comparison with the controls.

No lung or brain edema were reported in treated rats, for both doses. Microscopic alterations were dispersed in several tissues in control and treated groups. Extramedullary hematopoiesis was reported in the spleen of control and treated groups. Some dispersed focal nonsuppurative areas of pneumonitis were reported in lungs of rats from the control and treated groups. At the 6-month time point, dispersed microscopic alterations were observed in the spleen and the kidneys: in the spleen, extramedullary hematopoieses and pigmented areas were seen in control and treated groups, while in the kidneys, mild nephritis was evidenced in some animals.

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

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

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

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

No mortality occurred and no effects on body weight or body weight changes were noted during the study. Hemoglobin levels were reduced at 1 month. No effects were seen on the erythrocytes count, hematocrit, methemoglobin and prothrombin levels. T4 levels were reduced throughout the study, at both doses. The decrease was statistically significant at 1-month time points in animals exposed to 750 ppm and at the 6 months time point in both exposed groups. OCT levels increased at 1 and 3 months, at both dose levels, however the serum levels were inferior to control values at 6 months.

Thyroid gland weights were increased after 6 months of exposure. As no more information is available, it is supposed that this effect appeared at both doses. At the 1-month time point, modifications were seen in the lungs as focal aeras of mild to severe haemorrhage and congestion of the alveolar area and duct walls. Edema and sometimes necrosis were seen in the congestioned or bleeding areas. Lung edema was also reported in some animals. Nonsuppurative pericholangitis and nonsuppurative focal encephalitis were observed in control and exposed groups.

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

For a 90-d study, doses relevant for STOT RE classification are, approximatively, for a 6-h daily exposure: ≤ 0.2 mg/L/d for Cat. 1 and 0.2 ≤ C ≤ 1 mg/L/d for Cat. 2, respectively. The dossier submitter considers therefore this repeated dose toxicity study as relevant for STOT RE classification with the dose of 100 ppm. Dose of 750 ppm is outside the CLP guidance range for STOT RE classification and doses selction 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 46). Body weights were not affected in males but they were slightly higher than in controls in females exposed to 375 ppm (see Table 47). 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 48).

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

In a 2-year study in mice (NTP, 1997), B6C3F1 male and female mice were exposed during 2 years to vapours of nitromethane at doses of either 0, 188, 375 or 750 ppm (6 hours/day, 5 days/week). The doses of 0, 188, 375 and 750 ppm were approximatively equivaent to 0, 0.47, 0.94 and 1.87 mg/L, respectively. Mortality tended to be high in all dose groups, in both sexes, but the survival rate of females exposed to the highest dose was marginally greater than in other groups (see Table 51). 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 52). Coincidently with a swelling around the eyes and exophthalmos in exposed animals of both sexes, neoplasms of the Harderian gland were observed (see Table 54).

Histopathological findings show that nasal lesions were increased in exposed animals of both sexes (Table 53). 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 54. Liver tumours were seen only in females: adenoma rates (28 – 36, 51 – 61, 35, -38 and 70 – 81

%, for overall – terminal rates, at 0, 188, 375 and 750 ppm, respectively) and carcinoma rates (20 – 12, 29 – 21, 16 – 23 and 24 – 6 %, for overall – terminal rates, at 0, 188, 375 and 750 ppm, respectively).

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

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

Case reports

/

Data on 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 120). 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 120: Body weight data (in g)

	Main groups				Recovery groups	
Dose level (in mg/kg bw/d)	0	10	30	100	0	100
Males						
D 0	138	141	143	140	142	141
D 14	249	255	267	236	256	254
D 21	298	305	327	281	302	295
D 28	329	338	368	296	345	334
D 42	/	/	/	/	399	390
Females						
D 0	131	145	137	140	137	143
D 14	199	205	197	197	196	201
D 21	221	229	226	220	220	221

D 28	231	245	239	234	240	236
D 42	/	/	/	/	263	253

Significantly lowered hemoglobin and hematocrit values, erythrocyte count and significantly lowered white blood cell count were observed in females of the highest dose. In males, the methemoglobin was significantly increased at the low 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 121).

Table 121: Hematological findings

	Males						Females					
	Main groups				Satellite group		Main groups				Satellite group	
Dose level (in mg/kg bw/d)	0	10	30	100	0	100	0	10	30	100	0	100
Hb (g/dL)	14.7	14.9	15.1	14.0	15.6	16.4	14.9	14.3	14.2	14.1*	15.3	14.6
Ht (%)	43.2	43.9	44.2	42.3	44.6	46.4	43.6	42.4	41.6	40.2**	43.5	41.3*
RBC (10^{12}/L)	7.78	7.72	7.72	7.65	8.12	8.48	7.80	7.60	7.48	7.38*	7.88	7.64
WBC (10^9/L)	13.0	12.4	12.6	14.0	12.3	14.4	11.4	9.4	12.3	14.5*	11.9	10.3
Meth (%)	0.87	2.67*	0.94	1.19	0.54	1.12**	0.47	0.54	0.93	1.28	0.34	0.35
Lymph (10^9/L)	11.26	10.17	11.14	12.46	9.24	11.81*	9.35	8.06	10.94	12.67*	8.38	7.37
CT (s)	26	27	27	28	26	26	25	27	27	28*	25	26
Plt (10^9/L)	1102	1174	1220	1115	1304	1080**	1094	1156	1056	1264	1112	1140

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 19 (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 dehydratation 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 sloughting 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 significanty affected in females (see Table 80 and Table 81). At necropsy, organ weights were examined and revealed few significant changes (see Table 82). 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 83).

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

Case report

/

Table 122: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Respiratory tract				
Range-finding of the 28-day repeated dose toxicity study in Rat Oral route 1-nitropropane Anonymous 38, 1996	No effect observed in respiratory tract (however nasal cavity not examined)	14 D	/	/
Short-term repeated dose toxicity study in Rat Oral route 1-nitropropane Anonymous 38, 1996	No effect observed in respiratory tract (however nasal cavity not examined)	28 D	/	/
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in Rat Inhalation route 1-nitropropane Anonymous 37, 2003	Degeneration and inflammation of the olf. epith. at 100 ppm corresp. approx to 0.369 mg/L	Male: min. 28 D Female: ± 45 D	Male: ± 0.12 mg/L Female: ± 0.18 mg/L	STOT RE Cat. 1 As ≤ 0.2 mg/L
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	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

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

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
	Moderate degeneration olf. epith. + inflammation + moderate glandular hyperplasia already at 350 ppm (corresp. approx.. to 1 mg/L)	Terminal sacrifice: 93 D	1.0 mg/L	SOT RE Cat. 1 (borderline to Cat. 2)
2-year inhalation toxicity study in Rat Nitromethane NTP, 1997	No effect observed in respiratory tract	2 y	/	No classification
2-year inhalation toxicity study in Mouse Nitromethane NTP, 1997	≥ 188 ppm (cooresp. approx.. to 0.47 mg/L): sign increase degeneration olf. epith.	2 y	3.76 mg/L	No classification
Chronic inhalation toxicity study in Rat Nitroethane Anonymous 35, 1986	No effects observed	2 y	/	No classification
Blood				
Range-finding of the 28-day repeated dose toxicity study in Rat 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	Hematology not examined	16 D	/	/

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Nitromethane NTP, 1997				
16-day repeated dose toxicity study in Mouse Inhalation route Nitromethane NTP, 1997	Hematology not examined	16 D	/	/
13-week repeated dose toxicity study in Rat Inhalation route Nitromethane NTP, 1997	0.938 mg/L	13 W	0.938 mg/L	STOT RE 2
13-week repeated dose toxicity study in Mouse Inhalation route Nitromethane NTP, 1997	3.75 mg/L (extramedullary hematopoiesis in spleen)	13 W	3.75 mg/L	No classification But hematology not peformed
Sub-chronic repeated dose toxicity study in Rat Inhalation route Nitromethane Lewis *et al.*, 1977	1.875 mg/L	13 W	1.875 mg/L	No classification
Sub-chronic repeated dose toxicity study in Rabbit Inhalation route Nitromethane Lewis *et al.*, 1977	1.875 mg/L (Hb reduced at 1 month)	1 month	0.625 mg/L	STOT RE 2
13-week repeated dose toxicity study in Rat Inhalation route Nitroethane Anonymous 26, 1982	0.3 mg/L	Terminal sacrifice: 92 D	0.3 mg/L	STOT RE 2
13-week repeated dose toxicity study in Mouse Inhalation route Nitroethane Anonymous 26, 1982	3.0 mg/L	Interim sacrifice: ± 1 month	3.0 mg/L	No classification

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
		Terminal sacrifice: 93 D		
2-year inhalation toxicity study in Rat Nitromethane NTP, 1997	Hematology not examined	2 Y	/	/
2-year inhalation toxicity study in Mouse Nitromethane NTP, 1997	Hematology not examined	2 Y	/	/
Chronic inhalation toxicity study in Rat Nitroethane Anonymous 35, 1986	No effects observed However MetHb not examined	2 Y	/	/
Nervous system				
Range-finding of the 28-day repeated dose toxicity study in Rat Oral route 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	0.938 mg/L	16 D	0.16 mg/L	STOT RE 1

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
NTP, 1997				
16-day repeated dose toxicity study in Mouse Inhalation route Nitromethane NTP, 1997	3.75 mg/L	16 D	0.625 mg/L	STOT RE 2 (but only clinical signs observed)
13-week repeated dose toxicity study in Rat Inhalation route Nitromethane NTP, 1997	0.938 mg/L	13 W	0.938 mg/L	STOT RE 2
13-week repeated dose toxicity study in Mouse Inhalation route Nitromethane NTP, 1997	No effects observed	13 W	/	No classification
Sub-chronic repeated dose toxicity study in Rat Inhalation route Nitromethane Lewis *et al.*, 1977	No effects observed	13 W	/	No classification
Sub-chronic repeated dose toxicity study in Rabbit Inhalation route Nitromethane Lewis *et al.*, 1977	No effects observed	At 1 month	/	No classification
13-week repeated dose toxicity study in Rat Inhalation route Nitroethane Anonymous 26, 1982	No effects observed	Interim sacrifice: ± 1 month Terminal sacrifice: 92 D	/	No classification
13-week repeated dose toxicity study in Mouse Inhalation route Nitroethane	3.0 mg/L	Terminal sacrifice: 93 D	3.0 mg/L	No classification

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Anonymous 26, 1982				
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

<table>
<tr><th>Criteria for STOT RE 1</th><th>Criteria for STOT RE 2</th></tr>
<tr><td>“Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.

Substances are classified in category 1 for target organ toxicity (repeat exposure) on the basis of:
▪ Reliable and good quality evidence from human cases or epidemiological studies; or
▪ Observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.”

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

Table 3.9.2
<table><tr><td>Route of exposure</td><td>Units</td><td>Guidance value</td></tr></table></td><td>Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure.

Substances are classified in category 2 for target toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.”

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

Table 3.9.3
<table><tr><td>Route of exposure</td><td>Units</td><td>Guidance value range</td></tr><tr><td>Oral (rat)</td><td>mg/kg bw/d</td><td>10 < C ≤ 100</td></tr></table></td></tr>
</table>

Oral (rat)	mg/kg bw/d	C≤10	

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 123).

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 123: Summary data about respiratory tract in the subacute toxicity study

		Guidance value range for	DS's conclusion

		warranting classification	
1-Nitropropane			
Range-finding of the 28-day repeated dose toxicity study Oral route Rat (SD) 3/sex/dose 0, 10, 50 150 and 250 mg/kg bw/d 14 D of exposure Anonymous 38, 1996	No effects observed in respiratory tract However nasal cavity not examined	Cat. 2: > 60 and ≤ 600 mg/kg bw/d Cat. 1: C ≤ 60 mg/kg bw/d	No classification based on the result but nasal cavity not examined microscopically
Short-term repeated dose toxicity study Oral route Rat (SD) 5/sex/dose 0, 10, 30 and 100 mg/kg bw/d (and 2 recovery groups: 0 and 100 mg/kg bw/d) 28 D of exposure (Recovery period: 14 D) Anonymous 38, 1996	No effects observed in respiratory tract However nasal cavity not examined	Cat. 2: > 30 and ≤ 300 mg/kg bw/d Cat. 1: C ≤ 30 mg/kg bw/d	No classification based on the result but nasal cavity not examined microscopically
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Inhalation route Rat (SD) 12/sex/dose 0, 25, 50 and 100 ppm (± 0, 0.092, 0.184 and 0.369 mg/L) Males: minimum 28 D Females: ± 45 D Anonymous 37, 2003	Degeneration of the olf. epith. (multifocal) in 7 F (5 VS and 2 S) and in 2 M (1 VS and 1 S) at 100 ppm (not observed in the other groups) Degeneration olf. epith. with inflammation (focal) in 2 F (VS) at 50 ppm and in 2 F (S) at 100 ppm Degeneration olf. epith. with inflammation (multifocal) in 2 F (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	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	Degeneration and inflammation observed 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 ≤ 6 mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	Degeneration observed at doses within the range to classify in Cat. 1
16-day repeated dose toxicity study Inhalation route	1500 ppm: tachypnea in both sexes	Cat. 2: 1.2 < C ≤ 6 mg/L/6 h/d	Degeneration observed at doses within the

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	≥ 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. 1: C ≤ 1.2 mg/L/6 h/d	range to classify in Cat. 1
Nitroethane			
No subacute toxicity study available	/	/	/

Sub-chronic toxicity studies

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

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

Table 124: Summary data about respiratory tract in sub-chronic toxicity study

		Guidance value range for warranting classification	DS's conclusion
Nitromethane			
13-week repeated dose toxicity study Inhalation route Rat (F344) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	≥ 375 ppm: Degeneration of the olf. epith. in both sexes (in 0, /, 0, 9**, 10** and 10** M and in 0, 0, 1, 10**, 10** and 10** F) ≥ 750 ppm: Hyaline droplets olf. epith. (0, /, 0, 0, 1 and 8** M and 0, 0, 0, 0, 4* and 10** F)	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Sign increased inc. of degeneration olf. epith. at dose relevant to classify in Cat. 2
13-week repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure	≥ 375 ppm: Degeneration olf. epith in M (0, 0, 0, 10**, 10** and 10**) + Hyaline droplets olf. epith. (0, 0, 1, 10**, 10** and 10**) ≥ 188 ppm: Degeneration olf. epith in F (0, 0, 7**, 10**, 10** and 10**) + Hyaline droplets olf. epith. (0, 2, 9**, 10**, 10** and 10**)	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Increased inc. of degeneration olf. epith. at doses within the range to classify in Cat. 2

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 *et al.*, 1977	No sign. increased incidence of effect in respiratory tract. However, nasal cavity not examined microscopically	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d	No classification
Sub-chronic repeated dose toxicity study Inhalation route Rabbit (NZW) 15 M/dose 100 and 750 ppm (± 0.25 and 1.875 mg/L) 13 w of exposure Lewis *et al.*, 1977	≥ 100 ppm: at the 1-month time point, modifications in the lungs as focal aeras of mild to severe hemorrhage and congestion of the alveolar area and duct walls. Interstitial edema of the alveolar and alveolar duct walls and some degree of alveolar wall necrosis seen in the area of hemorrhage and congestion.	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d For the 1-month time point: 0.6 < C ≤ 3 mg/L/6 h/d	Indication of respiratory effects (after 1 month) at dose to classify in Cat. 1
Nitroethane			
13-week repeated dose inhalation toxicity study Inhalation route Rat (F344) 15/sex/dose 0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L) 92 D Anonymous 26, 1982	At interim sacrifice (5 animals/sex/group examined): ± 1 month Slight diffuse degeneration olf. epith. in 3 M at 350 ppm and in 5 M and 5 F at 1000 ppm Slight chronic active inflammation olf. epith in 1 F at 100 ppm, in 5 M and 1 F at 350 ppm and in 5 M and 5 F at 1000 ppm At terminal kill At 1000 ppm: Moderate diffuse degeneration olf. epith in 5 M and 5 F (out of 5/sex tested) (Slight in 1 M and 2 F at 350 ppm) + slight diffuse chronic active inflammation olf. epith. in 4 M and 5 F (out of 5 tested/sex) (also in 2 F at 350 ppm)	For interim kill: 30-day Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill (90-day) Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Effects already observed at doses within the range to classify in Cat. 2
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	At interim sacrificed (5 animals/sex/group examined): ± 1 month Moderate olf. epith. degeneration + inflammation in 3 M and 4 F at 350 ppm and in 4 M and 5 F at 1000 ppm Moderate glandular hyperplasia olf. epith. in 2 M and 4 F at 350 ppm and in 4 M and 4 F at 1000 ppm	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	Effects already observed at doses within the range to classify in Cat. 2

Anonymous 26, 1982	At terminal sacrifice (5 animals/sex/group examined) Moderate olf. epith. degeneration + inflammation in 4 M and 5 F at 350 ppm and in 5 M and 5 F at 1000 ppm Moderate glandular hyperplasia olf. epith. in 4 M and 5 F at 350 ppm and in 4 M and 5 F at 1000 ppm	For terminal kill (90-day) Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	

Chronic toxicity studies

Three chronic repeated dose toxicity studies are available (2 with nitromethane and 1 with nitroethane).
As observed in Table 125, no effect was observed in 2 of these studies. While, in one of the studies performed with nitromethane, degeneration of the olfactive epithelium was observed however at dose which does not warrant a classification.

Table 125: Summary data about respiratory tract in chronic toxicity study

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

Conclusion for respiratory tract:

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

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

- **Nervous system**

Sub-acute toxicity studies

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

As observed in Table 126, 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 126: 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 supproting 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)	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

28 D of exposure (Recovery period: 14 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 (± 0, 0.092, 0.184 and 0.369 mg/L) Males: minimum 28 D Females: ± 45 D Anonymous 37, 2003	In M: Sign ↑ rela brain weight at the highest dose	For 28 D of exposure Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: C ≤ 0.6 mg/L/6 h/d For ± 45 D of exposure Cat. 2: : 0.4 < C ≤ 2 mg/L/6 h/d Cat. 1: C ≤ 0.4 mg/L/6 h/d	Brain weight modified at dose supporting Cat 1 Only very low dose tested
Nitromethane			
16-day repeated dose toxicity study Inhalation route Rat (F344) 5/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	1500 ppm: hyperactivity at the beginning and hypoactivity and loss of coordination in hindlimbs at the end of the study ≥ 750 ppm: reduced myelin around sciatic nerve ≥ 375 ppm: sign. and DR increase inc. of sciatic nerve degeneration (in all animals at the 3 highest doses)	Cat. 2: 1.2 < C ≤ 6 mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	STOT RE Cat. 1 Sciatic nerve degeneration already observed at ≥ 0.938 mg/L
16-day repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	1500 ppm: reduced activity (sciatic nerve not examined)	Cat. 2: 1.2 < C ≤ 6 mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	STOT RE 2 (but only clinical signs)
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 127: Summary data on nervous system after sub-chronic exposure

		Guidance value range for warranting classification	DS's conclusion
Nitromethane			
13-week repeated dose toxicity study Inhalation route Rat (F344) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	1500 ppm: hindlimbs paralysis in all animals (starting from D 21) 750 ppm: hindlimbs paralysis in 1 M and 4 F (starting from D 63) ≥ 750 ppm: grip strength sign. reduced ≥ 375 ppm: sign. and DR increase inc. of sciatic nerve degeneration (in 5**, 10** and 10** M and in 8**, 10** and 10** F, resp. at 375, 750 and 1500 ppm) and spinal cord degeneration (in 9**, 10** and 10** M and in 2, 10** and 10** F, resp. at 375, 750 and 1500 ppm) + startle response amplitude ended to decrease	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	STOT RE Cat. 2 At 375 ppm (corresp. approx.. to 0.938 mg/L): sign. increase sciatic nerve and spinal cord degeneration + at the highest dose, hindlimbs paralysis observed after 21 D of exposure
13-week repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	No effects observed Neurobehavioral measurement not performed	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	No classification
Sub-chronic repeated dose toxicity study Inhalation route Rat (SD) 50 M/dose 100 and 750 ppm (± 0.25 and 1.875 mg/L) 13 w of exposure Lewis *et al.*, 1977	No effects observed	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d	No classification
Sub-chronic repeated dose toxicity study Inhalation route Rabbit (NZW) 15 M/dose	No effects observed	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d	No classification

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

Chronic toxicity studies:

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

Table 128: 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)	No effects observed	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	No classification

0, 94, 188 and 375 ppm (± 0, 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 (± 0, 0.47, 0.94 and 1.87 mg/L) 2 y of exposure NTP, 1997	No effects observed	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	No classification
Nitroethane			
Chronic inhalation toxicity study Inhalation route Rat (Long-Evans) 40/sex/dose 0, 100 and 200 ppm (± 0.31 and 0.61 mg/L) Anonymous 35, 1986	No effects observed	Cat. 2: 0.025 ≤ C ≤ 0.125 mg/L/d Cat. 1: ≤ 0.025 mg/L/d	No classification

Conclusion for nervous system:

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

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

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

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

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

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

- **Blood**

Sub-acute toxicity studies:

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

Table 129: Summary data on hematological effects after sub-acute exposure

		Guidance value range for warranting classification	DS's conclusion
1-Nitropropane			
Range-finding of the 28-day repeated dose toxicity study Oral route Rat (SD) 3/sex/dose 0, 10, 50 150 and 250 mg/kg bw/d 14 D of exposure Anonymous 38, 1996	At 150 and 250 mg/kg bw/d: clinical signs such as pallor of extremities, lethargy + pale kidneys Only at 250 mg/kg bw/d: pale liver and adrenals At 250 mg/kg bw/d: all animals died during the study	Cat. 2: > 60 and ≤ 600 mg/kg bw/d Cat. 1: C ≤ 60 mg/kg bw/d	Clinical signs at dose supporting Cat. 2
Short-term repeated dose toxicity study Oral route Rat (SD) 5/sex/dose 0, 10, 30 and 100 mg/kg bw/d (and 2 recovery groups: 0 and 100 mg/kg bw/d) 28 D of exposure (Recovery period: 14 D) Anonymous 38, 1996	In F: at 100 mg/kg bw/d: Sign. and DR ↓ of Hb, Ht and RBC (also observed in recovery group) MetHb: ↑ DR (not sign.)	Cat. 2: > 30 and ≤ 300 mg/kg bw/d Cat. 1: C ≤ 30 mg/kg bw/d	Sign. and DR hematological effects supporting Cat. 2
Combined repeated dose toxicity with the reproduction/developmental toxicity screening test Inhalation route Rat (SD) 12/sex/dose 0, 25, 50 and 100 ppm (± 0, 0.092, 0.184 and 0.369 mg/L) Males: minimum 28 D Females: ± 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 ≤ 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	Slight decrease MetHb in M Only very low doses tested
Nitromethane			
16-day repeated dose toxicity study	Not examined	Cat. 2: 1.2 < C ≤ 6	/

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		mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	
16-day repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) NTP, 1997	Not examined	Cat. 2: 1.2 < C ≤ 6 mg/L/6 h/d Cat. 1: C ≤ 1.2 mg/L/6 h/d	/
Nitroethane			
No subacute toxicity study available	/	/	/

Sub-chronic exposure:

Table 130: Summary data on hematological effects observed after a sub-chonic exposure

		Guidance value range for warranting classification	DS's conclusion
Nitromethane			
13-week repeated dose toxicity study Inhalation route Rat (F344) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	Concentration-dependent, microcytic responsive anemia Characterized by mild to moderate decreases in Ht and Hb values and minimal to moderate decreases in mean cell volume at all time points at ≥ 375 ppm Platelets count midly to markedly increased in all treated group MetHb increased in M at ≥ 375 ppm and in F at 750 ppm and 1500 ppm	Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Effects observed at doses supporting Cat. 2
13-week repeated dose toxicity study Inhalation route Mouse (B6C3F1) 10/sex/dose 0, 94, 188, 375, 750 and 1500 ppm (± 0, 0.235, 0.47, 0.938, 1.88 and 3.75 mg/L) 13 w of exposure NTP, 1997	Minimal extramedullary hematopoiesis in spleen at 1500 pm (in 0, 1, 0, 1, 2 and 10 M and in 0, 0, 0, 2, 3 and 9** F, resp. at 0, 94, 188, 375, 750 and 1500 ppm) Hematological examination not performed	Cat. 2: 0.2 < C ≤ 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 (± 0.25 and 1.875 mg/L) 13 w of exposure Lewis *et al.*, 1977	750 ppm: sign. Decrease in Ht and Hb MetHb not sign. modified	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d	No classification
Sub-chronic repeated dose toxicity study Inhalation route Rabbit (NZW) 15 M/dose 100 and 750 ppm (± 0.25 and 1.875 mg/L) 13 w of exposure Lewis *et al.*, 1977	Hb reduced at 1 month at the highest dose (no info for 100 ppm)	Cat. 2 for 13-week exposure: 0.2 < C ≤ 1 mg/L/6 h/d For the 1-month time point: 0.6 < C ≤ 3 mg/L/6 h/d	STOT RE 2
Nitroethane			
13-week repeated dose inhalation toxicity study Inhalation route Rat (F344) 15/sex/dose 0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L) 92 D Anonymous 26, 1982	1000 ppm: sign. increase in methemoglobin levels (associated with cyaniosis), in reticulocytes and Heinz bodies in blood associated with splenic congestion and extramedullary hematopoiesis Increase inc. of spleen darkness in all animals at 1000 ppm and in 9 M at 350 ppm Increase inc. of spleen congestion (in all M at ≥100 ppm and in 5, 4 and 5 F, resp. at 100, 350 and 1000 ppm) + extramedullary hematopoiesis (in all M at ≥100 ppm and in 1, 2 and 1 F, resp. at 100, 350 and 1000 ppm)	For interim kill: 30-day Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill (90-day) Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	Supporting Cat. 2 based on microscopic effects
13-week repeated dose inhalation toxicity study Mouse (B6C3F1) 5/sex/dose 0, 100, 350 and 1000 ppm (± 0, 0.3, 1.0 and 3.0 mg/L) 93 D Anonymous 26, 1982	MetHb sign increase at 1000 ppm in F Heinz bodies sign. ↑ in both sexes at 1000 ppm (tend to ↑ at low and mid doses) No splenic microscopic effects observed	For interim kill: 30-day Cat. 2: 0.6 < C ≤ 3 mg/L/6 h/d Cat. 1: ≤ 0.6 mg/L/6 h/d For terminal kill (90-day) Cat. 2: 0.2 < C ≤ 1 mg/L/6 h/d Cat. 1: ≤ 0.2 mg/L/6 h/d	No classification

Chronic exposure:

Table 131: Summary data on hematological effects after chronic exposure

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

Conclusion for blood:

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

The NTP paper describes the effects as "*exposure to nitromethane caused an exposure concentration-dependent, microcytic, responsive anemia in rats. The anemia was characterized by mild to moderate decreases in hematocrit values and hemoglobin concentrations, and the microcytosis was evidenced by minimal to moderate decreases in mean cell volume.*

- Conclusion

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

10.12.3 Conclusion on classification and labelling for STOT RE

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

10.13 Aspiration hazard

Not evaluated in this CLH dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this CLH dossier.

13 ADDITIONAL LABELLING

NA

14 ABBREVIATIONS

*	P<0.05
**	P<0.01
***	P<0.001
1-NP	1-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
Avg3	Average
B. or bilat.	Bilateral

Bili	Bilirubine
Bronch	Bronchiolar
BUN	Blood urea nitrogen
BW	Body weight
BWG	Body weight gain
CE	Cloning efficiency
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
Chrom	Chromosome
CMC	carboxymethylcellulose
Conc.	Concentration
Corresp.	Corresponding
CP	Cyclophosphamide
CT	Clotting time
D or d	Day
DMSO	dimethyl sulfoxide
DNA	Desoxyribonucleic acid
DR	Dose-related
DS	Dossier submitter
E.C.L.	Estrus cycle length
E. coli	Escherichia coli
ELISA	Enzyme-linked immunoabsorbent assay
Epith.	Epithelium
F	Female
FBW	Final Body Weight
Flam	Flammable
G or g	Gram
GD	Gestational day
GLP	Good laboratory practices
Gp	Group
GV	Guidance value
H or h	Hour
Hb	Hemoglobin
HCD	Historical control data
Hg	Mercury
HGPRT	Hypoxanthine-guanine phosphoribosyltransferase
Ht	Hematocrit
IC95	confidence interval 95%
Impl.	Implantation
Inc.	Incidence
Infla	Inflammation
IP	Intraperitoneal
K	Potassium
L.	Left
LC0	Lethal concentration 0%
LC50	Lethal concentration 50%

LC100	Lethal concentration 100%
LD0	Lethal dose 0%
LD50	Lethal dose 50%
Liq.	Liquid
LOAEC	Low observed adverse effect concentration
LOAEL	Low observed adverse effect level
Lymph	Lymphocyte
M	Male
max	Maximum
MCV	Mean cell volume
Met. Act.	Metabolic activation
MetHb	Methemoglobin
MHPG	3-Methoxy-4-hydroxyphenylglycol
Min	Minimum
MMC	Mitomycin C
MN	Micronuclei
MNBC	Micronucleated binucleated cells
Multifoc.	Multifocal
N or No or Nb	Number
NA	Not applicable
NC	Negative control
NCE	Normochromatic erythrocytes
ND or N.D.	Not determined
NE	Nitroethane
Neg	Negative
NM	Nitromethane
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOEC	No effect concentration
NTP	National toxicology program
Nucl.	Nucleated
NZW	New Zealand White
Olf.	Olfactory
OCT	Ornithine carbamyl transferase
O.E.	Olfactory epithelium
PC	Positive control
PCE	Polychromatic erythrocytes
PCV	Pack cell volume
Plt	Platelet
PND	Postnatal day
Pos	Positive
PROT	Protein
PT	Prothrombin
R.E.	Respiratory epithelium
RBC	Red blood cell
RCS	Relative cell survival

Rel	Relative
Repr.	Reproductive toxicity
Resp.	respectively
Resp. epith.	Respiratory epithelium
RPE	Relative plating efficiency
S.	slight
S. typh.	Salmonella typhimurium
SCE	Sister chromatid exchange
SD	Sprague-Dawley
SDH	Sorbitol Dehydrogenase
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 dose
STOT SE	Specific target organ toxicity – single dose
T3	Triiodothyronine
T4	Thyroxine
TCA Cycle	Tricarboxilic acid cycle
TG	Test guideline
Tot.	Total
Tox	Toxicity
V.S.	Very slight
WBC	White blood cell
Wk	week
Wng	Warning
Y	Year

15 ANNEXES

Confidential Annex to CLH report

Annex I to CLH report

16 REFERENCES

Beliles RP, Makris SL, Ferguson F *et al.,* 1978. Teratology study in mice subjected to inhalation of diethylhydroxylamine, nitroethane and diethylamine hydrogen sulfite. Environmental Research, 17, 165-176.

Bretherick L, 1990. Handbook of Reactive Chemical Hazards. 4th ed. Boston, MA: Butterworth-Heinemann Ltd., 1693.

Chemiekaarten, 12 ed., 1997.

Daubert, T.E. and R.P. Danner, 1989. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation, Washington, D.C.: Taylor and Francis.

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

Dequidt J, Vasseur P and Potencier, J., 1973. Etude toxicologique experimentale de quelques nitroparaffines. Bull Soc Pharm Lille, 29-35. English translation by Dr. PJ Baker Jr., IMC Chemical Group, Inc.

Fire Protection Guide to Hazardous Materials. 13 ed, (2002).

Heicklen J, Partymiller K and Kelly N., 1979. Teratology study in mice subjected to inhalation of diethylhydroxylamine, nitroethane and diethylamine hydrogen sulphite. Environ Res, 20, 450-454.

Hornfeldt C. and Rabe W., 1994. Nitroethane poisoning from an artificial fingernail remover. J Toxicol Clin Toxicol., 32(3), 321-324.

IPCS Inchem (nov 1998)

Kanada, M., Miyagawa, M., Sato, M., Hasegawa, H., and Honma, T., 1994. Neurochemical Profile of Effects of 28 Neurotoxic Chemicals on the Central Nervous System in Rats (1) Effects of Oral Administration on Brain Contents of Biogenic Amines and Metabolites. Industrial health, 32, 145-164.

Lide D. R., 1994. Handbook of Data on Organic Compounds Volume I. 3rd Edition. CRC Press, Inc. Boca Raton, Florida.

Machle, W., Scott, 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, No 8, 315-332.

Machle, W.; Scott, E. W.; and Treon, J. 1942. The metabolism of nitroparaffins. I. Recovery of nitroethane from the animal organism. Journal of Industrial Hygiene and Toxicology, Vol 24, 5−9.

Matsumoto, H.; Hylin, J. W., Miyahara, A., 1961. Methemoglobinemia in rats injected with 3-nitropropionic acid, sodium nitrite, and nitroethane. Toxicology and Applied Pharmacology, Vol 3, 493−499.

National Fire Protection Association, 2002. Fire Protection Guide to Hazardous Materials. 13 ed. Quincy, MA: National Fire Protection Association, 325-392.

Osterhoudt K.C., Wiley C., Dudley R., Sheen S. and Henretig F., 1995. Rebound severe methemoglobinemia from ingestion of a nitroethane artificial-fingernail remover. The Journal of Pediatrics, 126(5), 819-821.

Scott, E.W., 1942. The Metabolism of Nitroparaffins: II The Metabolic Products of Nitroethane. Journal of Industrial Hygiene and Toxicology, 24, 226-228/

Scott, E.W., 1943. The Metabolism of Mononitroparaffins: III The Concentration of Nitroethane, Nitrite and Nitrate in the Blood of Rabbits during Exposure by Inhalation and Oral Administration. Journal of Industrial Hygiene and Toxicology, 25, 20-25.

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

Snyder, R.; Andrews, L. S., 1996. Toxic effects of solvents and vapors. In Casarett and Doull's Toxicology, The Basic Science of Poisons, 5th ed.; Klaassen, C. D., Ed.; McGraw-Hill.

Yalkowsky, S.H., He, Yan, 2003. Handbook of aqueous solubility data: an extensive compilation of aqueous solubility data for organic compounds. CRC Press LLC, Boca Raton, Florida.