Agency technical report on the classification and labelling of: nitromethane

EC Number: 200-876-6
CAS Number: 75-52-5

March 2026

Contents

Brief summary

The conclusion of the Agency technical report is that nitromethane meets the classification criteria for:

Acute Tox. 4; H302 (Harmful if swallowed) with an ATE of 1450 mg/kg bw

Acute Tox. 4; H332 (Harmful if inhaled) with an ATE of 11 mg/L (vapour)

STOT RE 2; H373 (May cause damage to the respiratory tract and nervous system through prolonged or repeated exposure)

Carc. 1B; H350 (May cause cancer)

Repr. 1B; H360D (May damage the unborn child)

Is this in agreement with the RAC opinion? YES

At the time of publication, this mandatory classification and labelling (MCL) has not been agreed and/or adopted in Great Britain.

This is a targeted technical report which only considers acute toxicity via the oral and inhalation route, specific target organ toxicity – repeated exposure, carcinogenicity, germ cell mutagenicity and reproductive toxicity. These were the only hazard classes considered in the EU Committee for Risk Assessment (RAC) Opinion.

This substance has an existing MCL which includes Flam. Liq. 3 (H226). Flammable liquids and vapours are not assessed in this technical report, therefore, Flam. Liq. 3 (H226) should be retained in the GB MCL.

Introduction

Under Article 37 of the GB CLP Regulation[1], the Agency[2] is required to produce a technical report for each substance on which the Committee for Risk Assessment (RAC) of the European Chemicals Agency produces an opinion[3].

This technical report documents an independent scientific assessment, conducted by HSE technical specialists, of the classification and labelling of nitromethane.

Table 1. Information considered in the scientific assessment

Document	Included in assessment
EU CLH report	Yes
Annexes to the EU CLH report	Yes
RAC opinion	Yes
Background document	Yes
Information submitted during the EU public consultation process (RCOM table, including attachments)	Yes
RAC minority opinion(s)	Not applicable
Other information:	No

This information has been evaluated against the classification and labelling criteria set out in the GB CLP Regulation.

[1]The retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

[2] HSE acting in its capacity as the GB CLP Agency

[3] Under Article 37(4) of Regulation (EU) No 1272/2008 on classification, labelling and packaging of substances and mixtures

Overview of current and proposed classification and labelling

Table 2. Current and proposed classification and labelling

	Index No.	International Chemical Identification	EC No.	CAS No.	Classification		Labelling			Specific Concentration 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)		
GB MCL List entry	609-036-00-7	nitromethane	200-876-6	75-52-5	Flam. Liq. 3 Acute Tox. 4*	H226 H302	GHS02 GHS07 Wng	H226 H302		*	
EU dossier submitter's proposal	609-036-00-7	nitromethane	200-876-6	75-52-5	**Retain** Flam. Liq. 3 **Modify** Acute Tox. 4 **Add** Acute Tox. 3 Carc. 1B Repr. 1B STOT RE 2	**Retain** H226 H302 **Add** H331 H350 H360Df H373 (blood,respiratory tract and nervous system)	**Retain** GHS02 GHS07 **Remove** Wng **Add** GHS08 **Modify** Dgr	**Retain** H302 **Add** H331 H350 H360Df H373 (blood, respiratory tract and nervous system)		**Add** ATE (oral) = 1450 mg/kg bw ATE (inhalation) = 5.50 mg/L (vapour)	
EU RAC opinion	609-036-00-7	nitromethane	200-876-6	75-52-5	**Retain** Flam. Liq. 3 **Modify** Acute Tox. 4 **Add** Acute Tox. 4 Carc. 1B Repr. 1B STOT RE 2	**Retain** H226 H302 **Add** H332 H350 H360D H373 (respiratory tract and nervous system)	**Retain** GHS02 GHS07 **Remove** Wng **Add** GHS08 **Modify** Dgr	**Retain** H302 **Add** H332 H350 H360D H373 (respiratory tract and nervous system)		**Add** ATE (oral) = 1450 mg/kg bw ATE (inhalation) = 11 mg/L (vapour)	

	Index No.	International Chemical Identification	EC No.	CAS No.	Classification		Labelling			Specific Concentration 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)		
Agency technical report conclusion		nitromethane	200-876-6	75-52-5	**Retain** Flam. Liq. 3 **Modify** Acute Tox. 4 **Add** Acute Tox. 4 Carc. 1B Repr. 1B STOT RE 2	**Retain** H226 H302 **Add** H332 H350 H360D H373 (respiratory tract and nervous system)	**Retain** GHS02 GHS07 **Remove** Wng **Add** GHS08	**Retain** H302 **Add** H332 H350 H360D H373 (respiratory tract and nervous system)		**Add** ATE (oral) = 1450 mg/kg bw ATE (inhalation) = 11 mg/L (vapour)	
Resulting MCL entry on GB MCL list		nitromethane	200-876-6	75-52-5	Flam. Liq. 3 Acute Tox. 4 Acute Tox. 4 Carc. 1B Repr. 1B STOT RE 2	H226 H302 H332 H350 H360D H373 (respiratory tract and nervous system)	GHS02 GHS07 GHS08 Dgr	H226 H302 H332 H350 H360D H373 (respiratory tract and nervous system)		ATE (oral) = 1450 mg/kg bw ATE (inhalation) = 11 mg/L (vapour)	

Background

Active substance in Plant Protection Products: ☐

Active substance in Biocidal Products: ☐

Chemical registered under REACH: ☒

Nitromethane is a clear and colourless liquid at room temperature, with a vapour pressure of about 35 mmHg at 25.0°C. As nitromethane is a vapour, toxicity studies vapourised nitromethane into its gaseous form and is measured in ppm. However, concentrations are provided in mg/L.

Category approach and read-across – short chain nitroparaffins

In the initial CLH report, the dossier submitter (DS) evaluated the reliability of the studies assessed, providing Klimisch scores for each study. Studies with low reliability were considered in brief. In addition, the DS considered a read-across from members of the short chained nitroparaffins: nitroethane (EC/CAS No.: 201-188-9/79-24-3) and 1-nitropropane (EC/CAS No.: 203-544-9/108-03-2). This category approach was also taken by the OECD SIAM in 2010, noting that the substances are considered related due to their "*similarities in structure and in chemical and toxicological behaviour*". The category members are expected to have similar absorption, metabolism and excretion, resulting in the release of aldehydes and nitrite.

The classification proposals for acute oral and inhalation toxicity, carcinogenicity and reproductive toxicity were based on data from nitromethane. Available carcinogenicity studies on nitroethane and 1-nitropropane were assessed, but considered uninformative due to low dosing and low animal numbers. No prenatal developmental toxicity studies were performed on nitroethane and 1-nitropropane, and sperm parameters were not assessed in the available studies on nitroethane and 1-nitropropane. For STOT-RE, the classification proposal was based on an overall weight of evidence from all three substances.

RAC concluded that they did not support the read-across approach proposed by the DS for carcinogenicity and reproductive toxicity. The read-across from nitromethane (described as "*clear evidence of carcinogenicity and developmental toxicity*") to nitroethane and 1-nitropropane was not considered sufficiently justified, as the available data "*do not allow to observe a regular pattern in relevant toxicity studies on those hazard*

classes". Further, the ADME data were considered to be insufficient, and no information on a common mechanism of action was provided to strengthen the read-across justification. Therefore, these uncertainties prevent the application of read-across to carcinogenicity and reproductive toxicity.

However, RAC considered that the read-across was justified for nervous system toxicity; no toxicity was reported for nitroethane, whereas data on nitromethane and 1-nitropropane showed adverse effects. This is considered justified as the chain length of nitroethane (and its aldehyde) is between that of nitromethane and 1-nitropropane.

Overall, the Agency agrees with RAC's approach. The read-across approach appears justified for nervous system toxicity, however the read-across approach for carcinogenicity and reproductive toxicity is not supported.

Toxicokinetics – short chain nitroparaffins

Nitromethane, nitroethane, and 1-nitropropane are expected to have similar absorption, metabolism (common breakdown pathway to nitrite and corresponding aldehyde) and excretion patterns. Nitroethane has the most complete data set, therefore is used as the example for describing the breakdown pathway. Non-guideline, experimental data on TK were provided from *in vitro* and *in vivo* experiments on the metabolism of nitroethane in rabbits and Rhesus monkeys. Supportive evidence from QSAR predictions based on physico-chemical properties and systemic toxicity findings, as well as a review on the toxicity and metabolism of nitroalkanes and substituted nitroalkanes was provided.

Nitroethane is predicted to have low to moderate oral absorption through the human gastrointestinal tract (jejunum). This is consistent with the pKa of the compound (pKa= 8.8). Most of nitroethane will remain non-ionised in the jejunum, facilitating oral absorption and affording an estimated 100% oral bioavailability. The dermal permeability through the epidermis has been estimated to be 7.82×10^{-4}cm/h, consistent with low dermal toxicity in the rabbit. Inhalation of nitroethane is likely, due to relatively high vapour pressure (20.4 mm Hg at 25°C) and low octanol:air partitioning (K_{oa}=2.89).

The acidic nature of nitroethane (pH= 6.0 at 0.01 M solution), non-lipophilicity (log K_{ow}=0.45) and low plasma protein binding (~20%) means a moderate volume of distribution is estimated for nitroethane in humans. Oral administration of nitroethane to rats showed rapid detection of the parent compound in exhaled air, suggesting rapid absorption, distribution and elimination.

The metabolism of nitromethane in liver microsomes was studied in an *in vitro* study. Findings showed formation of a cytochrome p450-NO complex, catalisation by oxidised rat microsomes of the formaldehyde production in a non-NAPDH dependent reaction, and finally nitromethane was metabolised by cytochrome P450 into formaldehyde and nitrate (1:1) in the presence of NADPH and dioxygen. The parent compound was detected in the

liver and exhaled air from rats administered nitroethane via intraperitoneal injection- only nitrites were found in the heart, lungs, kidney, spleen and in urine. Similarly, most of the parent compound was rapidly excreted unchanged by the lungs, when rabbits were exposed to nitroethane either via oral or intravenous routes. The structure of nitroethane means it is expected that the nitro group will be reduced in the intestinal microflora to form the corresponding amine, which could be further deaminated by rat liver monoamine oxidase, to form the corresponding aldehyde. The aldehyde may be further metabolised to acetic acid by aldehyde dehydrogenase, therefore, acetic acid would be the major metabolite of nitroethane.

Un-metabolised nitroethane was rapidly excreted through the lungs, and remaining nitroethane will be rapidly metabolised into acetic acid. As acetic acid is more water soluble than the parent compound, it is expected to be rapidly excreted primarily in the urine. Similarly, nitrite has an average plasma elimination half-life of 21-35 mins in humans, so is expected to undergo rapid excretion.

Scientific assessment of the physical, human health and environmental hazard classes

Physical Hazards

Not assessed in the CLH report or RAC opinion.

Health Hazards

Acute Toxicity

Classification agreed by RAC:

Acute toxicity – oral route

Several studies with nitromethane in rats, mice, rabbits and dogs were available for the assessment of acute oral toxicity. None of the studies were performed in accordance with an existing guideline nor were they GLP-compliant.

As part of the first rat study (Anonymous, 1980), a probe study administered nitromethane to SD rats (5/dose) at doses of 0, 500, 1000, 1500 and 2000 mg/kg bw via oral gavage. Mortality was observed from 1000 mg/kg bw (3/5), and all rats (5/5) in the 2000 mg/kg bw dose group died within 24h of exposure. The main study exposed rats (10/sex/dose) to 0, 600, 800, 1000, 1400 and 1800 mg/kg bw nitromethane. All animals died in the 1800 mg/kg bw group. Mortality was observed in both sexes exposed to 1400 mg/kg bw (5 females, 3 males), whereas no deaths were reported in the 600, 800 or 1000 mg/kg bw groups. As a result of the deaths at 1800 mg/kg bw, a second experiment with 0 and 1600 mg/kg was conducted; 6/10 males and 8/10 females died at 1600 mg/kg bw. The acute oral LD_{50} was calculated to be 1506 mg/kg bw in males, 1449 mg/kg bw in females, and a combined LD_{50} (both sexes) of 1478 mg/kg bw.

A second rat study (Anonymous, 1960) with limited study information was not considered reliable by RAC. The acute oral LD_{50} was calculated as 1210 mg/kg bw.

In a study with white male mice (Weatherby, 1955), an aqueous solution containing 5% nitromethane was administered via gavage at doses of 1200 (5 males), 1500 (10 males) and 1800 (5 males) mg/kg bw. Mortality was observed from 1200 mg/kg bw (1/5) as well as at the mid dose of 1500 mg/kg bw (6/10) and at the top dose of 1800 mg/kg bw (4/5). The acute oral LD_{50} was estimated to be 1440 mg/kg bw.

A study in dogs (Weatherby, 1955) administered nitromethane in methylcellulose (20%) at doses of 125, 250, 500, 1000 or 1500 mg/kg bw. Group sizes varied between 2-6 dogs. In the 125 mg/kg bw group (5 dogs), one dog was euthanised at 24, 48 and 72h respectively with no reason specified; the remaining two dogs survived in good health. At 250 mg/kg bw, dogs died within 30h or were killed due to moribund state. In animals exposed to 1500 mg/kg bw, necropsy revealed effects on the kidneys and liver. The acute oral LD_{50} was estimated to be between 500-1000 mg/kg bw, although due to numerous reporting deficiencies, RAC considered this difficult to confirm. It is unclear whether this was calculated based on a corrected level of exposure (from 20% nitromethane solution). RAC considered this study should not be used for classification purposes.

Last, in an acute oral toxicity study in rabbits (Machle *et al.,* 1940), undiluted nitromethane was administered via gavage. Doses were not specified, but doses of at least 750 and 1000 mg/kg bw were used. After 20-40 mins of exposure, progressive weakness, unsteadiness and incoordination, ataxia and respiration modifications were reported. The acute oral LD_{50} was reported to be between 750 and 1000 mg/kg bw, with no further information.

RAC concluded that based on the derived LD_{50} from the reliable acute oral toxicity study in rats (1450 mg/kg bw, female rats), classification under category 4 (300 ≤ ATE ≤ 2000 mg/kg bw) for acute oral toxicity is warranted.

Acute toxicity – dermal route

Not assessed in the CLH report or RAC opinion.

Acute toxicity – inhalation route

Several acute inhalation toxicity studies with nitromethane in rats, rabbits and guinea pigs were available. All studies were noted to have limitations, including poor reporting and lack of guideline design/GLP. The DS included a read-across to 1-nitropropane, however, RAC rejected this read-across.

In an acute inhalation tox study (Anonymous, 1956), rats (n=10) were exposed whole body to 12.75 mg/L of nitromethane for one hour. All animals were reported to survive. The LC_0 (1h) was converted to an LC_0 (4h) of 3.2 mg/L.

In a second rat study (Dequidt *et al.,* 1973), Wistar rats (n=8-10, unspecified if per dose or total) were exposed to nitromethane vapour at 500, 2500 and 13000 ppm (equivalent to 1.25, 6.25 and 32.5 mg/L) for 6 hours per exposure. Total number of exposures varied between doses, with the top dose group experiencing a single exposure, whereas the animals at 2500 ppm experienced 4 exposures, and the 500 ppm animals were exposed 5 days/week for 3 weeks. At 13000 ppm (32.5 mg/L), all animals died after 6 h of exposure. At 2500 ppm, all animals died after the 4th day, and all animals in the 500ppm group

survived. RAC noted the contradicting information on the number of exposed animals for each group, meaning that an LC_{50} cannot be derived. The LC_{100} (6h) was determined to be 13000 ppm/32.5 mg/L, which can be converted to an LC_{100} (4h) of 48.75 mg/L.

In Machle *et al.,* (1940), rabbits (2/dose) and guinea pigs (2/dose) were exposed to nitromethane vapour. Exposure duration varied by dose, with exposure concentrations ranging between 0-50000 ppm (0-125 mg/L), and time ranging between 1 to 140h. All rabbits died at 10000 ppm (equivalent dose: 25 mg/L) after 6 hours of exposure, whereas no animals died at the same dose within 3 h of exposure. One death was observed at 5000 ppm (12.5 mg/L) after 6 hours. The LC_{50} was determined to be 5000 ppm, or 12.5 mg/L. In guinea pigs, all animals died after 1 hour of exposure at 50000 ppm (125 mg/L) and 30000 ppm (75 mg/L). At 10000 ppm (25 mg/L), all animals died after 6h of exposure, and one animal died in the 5000 ppm (12.5mg/L) group after 6h of exposure. The LC_{50} (6h) was determined to be 5000 ppm, or 12.5 mg/L. A converted LC_{50} (4h) of 18.75 mg/L for both rabbits and guinea pigs was derived.

RAC noted that it was not possible to derive an LC_{50} based on the acute inhalation studies in rats. Classification is based on the studies in rabbits and guinea pigs, taking into account the poor reporting and weaknesses in study design. The derived LC_{50} (4h) of 18.75 mg/L warrants classification under category 4 ($10.0 < ATE \leq 20.0$ mg/L) for acute inhalation toxicity (vapours). A converted acute toxicity point estimate of 11 mg/L is used as the ATE.

RAC concluded that classification for acute toxicity (inhalation route), category 4 with an ATE of 11 mg/L is warranted, diverging from the DS proposal of category 3 (ATE= 5.50mg/L).

Classification proposed by the Agency:

Acute toxicity – oral route

The Agency agrees with RAC's conclusion on the classification. Classification for acute toxicity via the oral route as **Acute Tox. 4; H302 (Harmful if swallowed) with an ATE of 1450 mg/kg bw** is warranted.

Acute toxicity – dermal route

Not assessed in the CLH report or RAC opinion.

Acute toxicity – inhalation route

The Agency agrees with RAC's conclusion on the classification. Classification for acute toxicity via inhalation as **Acute Tox. 4; H332 (Harmful if inhaled) with an ATE of 11 mg/L (vapour)** is warranted.

Specific target organ toxicity – single exposure (STOT SE)

Not assessed in the CLH report or RAC opinion.

Skin corrosion/irritation

Not assessed in the CLH report or RAC opinion.

Serious eye damage/irritation

Not assessed in the CLH report or RAC opinion.

Respiratory sensitisation

Not assessed in the CLH report or RAC opinion.

Skin sensitisation

Not assessed in the CLH report or RAC opinion.

Specific target organ toxicity – repeated exposure (STOT RE)

Classification agreed by RAC:

RAC utilised an overall weight of evidence approach using data from nitromethane, nitroethane and 1-nitropropane for the assessment of STOT-RE. These studies are summarised below.

Rat studies - nitromethane

One oral toxicity and four inhalation toxicity studies in the rat were available for assessment.

Oral: In a non-guideline, non-GLP study by Weatherby *et al.* (1955), male Albino rats (10/dose) were exposed to nitromethane (purity unknown) via drinking water at concentrations of 0, 0.1, 0.25% (equivalent doses: 0, 150, 285 mg/kg bw) for 15 weeks. At 0.25%, 3/10 animals died and at 0.1%, 4/10 animals died. Surviving animals reported a

decrease in body weight, alongside changes in liver (at 0.25% and 0.1%) and spleen (at 0.25% only) cells. RAC did not consider this study to be relevant for classification, as the tested doses exceeded guidance values for STOT-RE classification via the oral route.

Inhalation: Three studies were conducted by NTP (1997), ranging from 16 days to 2 years of exposure. Studies were conducted similarly to guidelines (OECD TG 413/451).

In the 16-day and 13-week studies (OECD TG 413), F344 rats were exposed to nitromethane vapours (purity: >98%) at concentrations of 0, 94, 188, 375, 750, 1500 ppm (corresponding to 0, 0.235, 0.47, 0.938, 1.88, 3.75 mg/L) for 6h/d and 5d/week. In the 16-day study, degeneration of the sciatic nerve and minimal-mild degeneration of the olfactory epithelium were observed from 375 ppm (=0.938 mg/L) in both sexes, with severity increasing with dose. Rats exposed to 750 and 1500 ppm were reported to have reduced myelin around the sciatic axon.

Similarly, in the 13-week study, degeneration of the sciatic nerve and olfactory epithelium was observed from 375 ppm (=0.938 mg/L) in both sexes, as well as spinal cord degeneration in males from 375 ppm and from 750 ppm in females. Haematological effects were also observed within both sexes. In males, haematocrit (Ht) and haemoglobin (Hb) levels were significantly decreased from 375 ppm and above, as well as an increased red blood cell (RBC) count from 94 ppm (=0.235 mg/L) in week 13. Methaemoglobin (MetHb) levels were significantly increased at 188 ppm (=0.47 mg/L) and above. In females, Ht and Hb levels were significantly decreased from 188 ppm, and MetHb increased from 750 ppm. RBC count increased and reached significance at some time points, but lacked a clear dose-response. RAC considered that the adverse haematological effects were corroborated by the observed dose-dependent bone marrow hyperplasia reported in females from 375 ppm, and in males from 750 ppm.

In the 2-year study, F344 rats were exposed to vapours of nitromethane at concentrations of 0, 94, 188 and 375 ppm (corresponding to 0, 0.235, 0.47, 0.94 mg/L), for 6h/d and 5d/week. Mortality was relatively high in all dose groups in both sexes (38, 28, 40 and 42 % of M and 50, 44, 48 and 28 % of F at each dose respectively) with no dose response. No non-neoplastic effects were observed at necropsy or in the microscopic and histopathological examinations up to the highest concentration. No further investigations were reported.

A fourth sub-chronic inhalation study by Lewis *et al.* (1977, non-guideline, non-GLP) exposed male SD rats to 100 and 750 ppm nitromethane (corresponding to 0.25 and 1.875 mg/L) for 7h/d, 5d/week for between 13-24 weeks. At 750 ppm (1.875 mg/L), Ht levels were significantly decreased, and Hb levels significantly increased from day 10 onwards, but without a clear dose-response relationship. RBC counts were also increased on day 2, but decreased from day 10 and after 3 months. No changes in MetHb was observed. No treatment-related effects were observed at 100 ppm (0.25 mg/L). As the effects were

mainly observed at 1.875 mg/L which exceeds guidance values for STOT-RE classification, RAC did not consider the study relevant for classification. However, they noted that this study supports identification of the blood as a target organ.

Mouse studies - nitromethane

Inhalation: Three studies were conducted by NTP (1997) in the mouse, ranging from 16 days to 2 years of exposure. Studies were conducted similarly to guidelines (OECD TG 413/451).

In the 16-day and 13-week studies, B6C3F1 mice (10/sex/group) were exposed to nitromethane vapour at concentrations of 0, 94, 188, 375, 750 or 1500 ppm (equivalent: 0, 0.235, 0.47, 0.938, 1.88, 3.75 mg/L) for 6h/d and 5d/week. In the 16-day study, degeneration of the olfactory epithelium was observed in both sexes from 375 ppm (0.938 mg/L) and above, with the severity graded minimal in males, and minimal-mild in females.

Similarly, in the 13-week study, mild degeneration of the olfactory epithelium was observed in both sexes from 375 ppm and above. In the 188 ppm (0.47 mg/L) group, 7/10 females were reported with minimal degeneration of the olfactory epithelium and at higher doses, all animals were affected to a mild degree.

In the 2-year study, mice (50/sex/group) were exposed to nitromethane at concentrations of 0, 188, 375, or 750 ppm (equivalent to: 0, 0.47, 0.94, 1.87 mg/L) for 6h/d, 5d/week. Degeneration of the olfactory epithelium was significantly increased at 188 ppm (0.47 mg/L) and above in both sexes, ranging from minimal to moderate in severity. At 375 ppm and above, minimal to mild olfactory epithelial metaplasia was observed in both sexes. Minimal-mild respiratory epithelial degeneration was significantly increased at 188 ppm and above in females, and from 375 ppm and above in males.

RAC considered that these studies support the identification of the respiratory tract as a target organ.

Rabbit studies - nitromethane

Inhalation: In a rabbit sub-chronic repeated dose toxicity study by Lewis *et al.* (1977), male NZW rabbits were exposed to 100 and 750 ppm (equivalent to 0.25 and 1.875 mg/L) nitromethane vapours for 13-24 weeks. The study was not conducted in accordance with guidelines or GLP. No mortality, clinical signs of toxicity, or treatment-related effects on haematological parameters were observed. As the effects did not reach significance or show a dose-response, RAC did not consider them to support classification for STOT-RE.

Rat studies - nitroethane

Inhalation: Two studies were available for the assessment of nitroethane toxicity in rats; one 13-week repeated dose toxicity study with a corresponding dose range finder

(Anonymous, 1982; OECD TG 413, GLP) and a 2-year chronic toxicity study (Anonymous, 1986; similar to OECD TG 454).

In the range finding study, F344 rats (5/sex/group) were exposed to nitroethane up to 4000 ppm (corresponding to 12.0 mg/L) for 4 days. All animals died at the top dose after 2 exposures; the study author proposed that the deaths were caused by hypoxia secondary to methaemoglobinemia. At 350 ppm (1.0 mg/L) and above, cyanosis (i.e. a manifestation of the MetHb effect) and hyperaemia of the nasal turbinates were observed.

In the 13-week study, rats were exposed to nitroethane vapours via inhalation at concentrations of 0, 100, 350 and 1000 ppm (equivalent to 0, 0.3, 1.0 and 3.0 mg/L) for 6h/d, 5d/week for either 30 days (5/sex/conc, interim sacrifice) or 92 days (10/sex/conc, terminal sacrifice).

At 1000 ppm, decreases in body weight gain were observed. Growth retardation was reported in the 350 and 1000 ppm groups for both sexes, with statistically significant body weight decreases during the last month of the study.

Haematological effects observed in this study included an increase in MetHb levels from 350 ppm in both sexes after 92 days of exposure. Significant increases in reticulocyte count and Heinz bodies were observed at 1000 ppm for both sexes. Other haematological effects observed included changes to packed cell volume (PCV), RBC count, white blood cell (WBC) count and Hb, but without clear dose-response and/or statistical significance. Adverse effects on the blood were observed in parallel with associated clinical signs, such as cyanosis and dull and dark red eyes. Other associated effects included splenic congestion and extramedullary haematopoiesis, particularly within males, from 100 ppm. Degeneration of the olfactory epithelium was observed from 350 ppm and above in both sexes, with severity (slight to moderate) increasing with dose.

In the 2-year chronic inhalation toxicity study (Anonymous, 1986), Long-Evans rats (39-41/sex/group) were exposed to nitroethane vapours at 0, 100 or 200 ppm (equivalent to 0, 0.31, 0.61 mg/L) for 7h/day, 5d/week. No treatment-related mortality was observed. A statistically significant decrease in body weight was reported at 100 ppm in males, and at 200 ppm in females. A slight, statistically significant increase in total protein and blood urea nitrogen was reported in females in the 200 ppm group. No further effects were observed on haematological or histopathological parameters, although RAC noted that MetHb was not analysed.

Mouse studies - nitroethane

Inhalation: In a 13-week repeated dose toxicity study (Anonymous, 1982; OECD TG 413, GLP), B6C3F1 mice (5/sex/group) were exposed to nitroethane vapours at concentrations of 0, 100, 350 and 1000 ppm (equivalent to: 0, 0.3, 1.0 and 3.0 mg/L) for 6h/day, 5d/week for either 30 days (interim sacrifice) or 93 days (terminal sacrifice).

At terminal sacrifice, MetHb levels were statistically significantly increased at 350 ppm (1.0 mg/L) and above in both sexes. In a time-sequenced analysis of MetHb (at 30m, 4h, 19h post exposure), MetHb levels decreased over time, and after 19h were comparable to controls in males, but remained statistically significantly higher in top dose (1000 ppm) females. Increases in reticulocyte count from 350 ppm occurred in both sexes, although statistical significance was only reached in females. Heinz bodies were statistically significantly increased at 1000 ppm (3.0 mg/L) for both sexes. Other haematological effects, such as changes to PCV, RBC count and Hb were observed, RAC noted that these changes were within the normal variability for the B6C3F1 mouse.

Slight to moderate degeneration of the olfactory epithelium with inflammation was observed from 350 ppm and above in both males and females, alongside moderate olfactory epithelial hyperplasia.

Rat studies – 1-nitropropane

Oral: One short-term repeated dose toxicity study with corresponding range finder (Anonymous, 1996, Japanese guideline, GLP) was available for assessment of 1-nitropropane toxicity via oral route. In the range-finding study, SD rats (3/sex/dose) were exposed via oral gavage to 0, 10, 50, 150 or 250 mg/kg bw/d of 1-nitropropane for 14 days. Severe clinical signs were observed from 150 mg/kg bw/d, including ataxia, pallor, tremors, lethargy and decreases in respiratory rate. All animals were killed in extremis at 250 mg/kg bw/d between day 4 to 9.

In the main study, SD rats (5/sex/dose) received 0, 10, 30, 100 mg/kg bw/d of 1-nitropropane via oral gavage for 28 days. Two satellite groups received either 0 or 100 mg/kg bw/d for 28 days, with a recovery period of 14 days. In the main groups, MetHb levels increased in both sexes, although statistical significance was only observed in males at 10 mg/kg bw/d. In the satellite group males, a statistically significant increase was observed at 100 mg/kg bw/d, which was comparable to the % MetHb observed in the main group (main: 1.19% vs. satellite: 1.12%). Satellite group female MetHb levels were comparable between 0 and 100 mg/kg bw/d.

In the main group, females in the 100 mg/kg bw/d group had statistically significant decreases in Hb, Ht, RBC count, and an increase in WBC and clotting time. These effects were not replicated in the satellite group, with the exception of a statistically significant decrease in Ht levels at 100 mg/kg bw/d. A statistically significant decrease in platelet count was observed in males at 100 mg/kg bw/d but only within the satellite group. RAC considered that this study supports the identification of the blood as a target organ.

Inhalation: In a combined repeated dose/reproductive toxicity study (Anonymous, 2003; OECD TG 422, GLP), SD rats were exposed to 1-nitropropane vapours at concentrations of 0, 25, 50, 100 ppm (equivalent to 0, 0.092, 0.184, 0.369 mg/L) for a minimum of 28 days in males, and for approx. 47 days in females. Histopathological examination showed slight

degeneration of the olfactory epithelium in both sexes at 100 ppm. This study was considered to support the identification of the respiratory tract as a target organ.

Other studies

Four additional studies on nitroethane were presented in the CLH, including two human case study reports, an oral neurotoxicity study in rats, and a hepatotoxicity study in mice.

Additional animal studies were disregarded by RAC; in the neurotoxicity study by Kanada *et al.* (1994), rats were exposed to 275 mg/kg bw of nitroethane via oral gavage (single exposure), before examination of neurochemicals in the brain. The study was disregarded as it was not possible to differentiate whether the observed effect was a direct effect of nitroethane or indirectly due to elevated MetHb levels. The hepatotoxicity study in mice (Dayal *et al.* 1989) administered nitroethane to mice via intraperitoneal injection, but had clear reporting deficiencies.

In one human case study, Hornfeldt and Rabe (1994) reported a 20-month old boy who ingested < 30mL of 100% nitroethane in nail polish remover. Cyanosis and increased levels of MetHb (39%) were reported. In the second case study, Osterhoudt *et al.* (1995) reported a case of a 13month old girl who ingested nail polish remover containing 100% nitroethane, with a max of 90mL missing from the bottle. Signs of cyanosis, lethargy, tachypnea, and elevated MetHb (53%) was observed. As observed in animal studies, cyanosis and increases in MetHb confirm human relevance. However, RAC considered that the limitations of the studies, poor reporting, and single exposure mean these studies would not be sufficient for classification under STOT-RE.

RAC conclusions on classification

RAC identified three target organs from the studies presented; blood, respiratory tract and nervous system.

Blood: No studies supporting STOT-RE 1 were identified. The following studies were considered of relevance for STOT-RE 2 classification:

- Nitromethane: 13-week inhalation study in rats (NTP 1997). At 0.938 mg/L, an increase in MetHb (M: 13%, F: 5%) observed in parallel with decreases in Ht (4-5%, both sexes) and Hb (3-4%, both sexes). Bone marrow hyperplasia was also observed at this concentration (6/10 females).
- Nitroethane: 13-week inhalation study in rats (Anonymous, 1982). Clinical signs of cyanosis, dull red eyes at 1.0 mg/L and above. However, effects were not distinctive and disappeared within 19h of exposure. Further, haematological effects such as reduced RBCs, reticulocytes and Heinz bodies were increased above 1.0 mg/L. This was considered similar to the 13-week mouse study (Anonymous, 1982).

- 1-nitropropane: 28-day oral study in rats (Anonymous, 1996). Decreased levels of Ht (8%), Hb (5%), RBC (5%) and increased WBC (27%) in females at 100 mg/kg bw/day. Upon extrapolation to the equivalent dose in a 90-day study, an effective dose of 33 mg/kg bw/d is obtained.

RAC noted that adverse effects were reported within the GV range for STOT RE 2 (inhalation (rat) vapours, 90d exposure: 0.2 < C ≤ 1.0 mg/L for nitromethane and nitroethane; oral (rat), 90d exposure: 10 < C ≤ 100 mg/kg bw/day for 1-nitropropane). However, RAC considered that the observed effects were not considered to be sufficiently significant or severe; therefore, no classification is warranted for effects on the blood.

Respiratory tract: Several studies with durations shorter or longer than 90 days were identified by RAC as relevant for classification. Studies with shorter durations supported classification as STOT RE 1, whereas longer exposure duration warranted no classification:

- Nitromethane: 16-day inhalation toxicity studies in rats and mice (NTP, 1997). Minimal to mild degeneration of the olfactory epithelium observed at 0.938 mg/L in both sexes. Extrapolation of the study duration to 90 days suggests that effects would be seen at doses supportive of STOT-RE 1.
- Nitromethane: 2-year inhalation toxicity study in mice (NTP, 1997). Minimal to moderate degeneration of the olfactory epithelium observed at 0.47 mg/L. Extrapolation of the study duration to 90 days resulted in an effective concentration outside of the guidance values, therefore this study does not support classification.
- 1-nitropropane: combined repeat dose/reproductive toxicity study (Anonymous, 2003) in rats. Very slight to slight degeneration of the olfactory epithelium observed at 0.369 mg/L in both sexes. Extrapolation of the study duration to 90 days results in effects supportive of STOT-RE 1.

Several 90-day studies were available. RAC gave more weight to these studies, compared to those described above:

- Nitromethane: In the 13-week inhalation toxicity studies in rats and mice (NTP, 1997), minimal to mild degeneration of the olfactory epithelium at 0.938 mg/L and above was observed in rats, and minimal degeneration at 0.47 mg/L and above in mice.
- Nitroethane: in the 13-week inhalation toxicity studies in rats and mice (Anonymous, 1982), slight to moderate degeneration of the olfactory epithelium was observed at 1.0 mg/L.

RAC concluded that the observed effects within the guidance value range for STOT-RE 2, as observed in the 90-d studies, were sufficiently significant/severe enough to warrant classification.

Nervous system: Two studies with a shorter than 90-day duration relevant for classification were identified by RAC.

- Nitromethane: 16-day inhalation toxicity study in rats (NTP 1997). Minimal to moderate degeneration of the sciatic nerve at 0.938 mg/L in both sexes. At 1.88 mg/L and 3.75 mg/L, the myelin around sciatic axons were reduced. Upon extrapolation of the study duration to 90 days, the effects occurred at doses supporting classification as STOT RE 1.
- 1-nitropropane: 14-day oral toxicity study in rats (Anonymous 1996). Ataxia, pallor of extremities, tremors, loss of righting reflex, lethargy, decreased respiratory rate, etc. were observed at 150 mg/kg bw/d and above. Upon extrapolation of the study duration to 90 days, the effects occurred at doses supporting classification as STOT RE 2.

An additional 13-week inhalation toxicity study in rats (NTP 1997) with nitromethane was available; this was given more weight by RAC compared to the 16-day study.

- Nitromethane: in the 13-week rat study, minimal to mild degeneration of the sciatic nerve and lumbar spinal cord were observed at 0.938 mg/L. At this concentration, 5/10 males and 8/10 females showed degeneration of the sciatic nerve, and 9/10 males and 2/10 females showed degeneration of the spinal cord. At higher concentrations, all animals were affected.

RAC concluded that the observed effects occurring at doses within the guidance value range for STOT RE 2 were sufficiently significant/severe to warrant classification.

RAC concluded that classification as STOT RE category 2 (respiratory tract, nervous system) was warranted.

Classification proposed by the Agency:

The Agency agrees with RAC's conclusion on the classification. Classification of **STOT RE 2 (H373; respiratory tract, nervous system)** for nitromethane is warranted.

Germ cell mutagenicity

Classification agreed by RAC:

In the CLH proposal, available *in vitro* and *in vivo* studies on nitromethane, nitroethane and 1-nitropropane performed in various experimental systems were presented. No studies on germ cells were available for any of the category members. The DS provided assessment of the reliability of the studies, with a Klimisch score for those included.

Nitromethane gave consistently negative results in *in vitro* bacterial mutagenicity assays, *in vitro* mammalian tests for sister chromatid exchanges and chromosomal aberrations. It did not induce micronuclei *in vitro* Syrian hamster embryo cells or *in vivo* in mice. However, a positive response was observed in a cell transformation assay in Syrian hamster embryo cells (Kerckeart *et al.* 1996). As this test responds to different mechanisms, including non-mutagenic mechanisms, and the *in vitro* micronucleus test was negative, it was concluded that this positive result was likely induced by non-mutagenic mechanisms.

RAC also considered the studies on nitroethane and 1-nitropropane in their assessment - the details of these studies can be found in the respective technical reports.

A summary of the nitromethane studies considered as part of the RAC opinion is provided in table 3 below:

Table 3: Summary of *in vitro* and *in vivo* data on nitromethane for assessment of germ cell mutagenicity

***In vitro* data - Nitromethane**			
Method, guideline, deviations (if any)	**Test substance and dosing information**	**Observations, limitations**	**Reference**
In vitro gene mutation test in bacteria OECD TG 471, non-GLP Deviation: 4 instead of 5 strains Reliability score: 2 (according to registration dossier)	Nitromethane (purity: >99%) Pre-incubation test Strains: *S. typh* (TA98, TA100, TA1535 and TA1537) Concs: 100, 333.3, 1000, 3333.3 and 10 000 µg/plate ± S9 Vehicle: DMSO	**Negative** No significant increase in frequency of revertant colonies up to 10mg/plate, ± S9. Cytotoxicity at the highest conc for TA100 only. Limitations: unclear whether protocol had been adapted for volatile compounds and, consequently, it remained unknown to which concentrations cells had been exposed.	Mortelmans *et al*. 1986

In vitro gene mutation test in bacteria Prior to OECD TG 471, GLP Reliability score: 2 (according to registration dossier, but study not available to DS. Interpret with caution.)	Nitromethane (no purity data) Strains: *S. typh* (TA98, TA100, TA1535, TA1537 and TA1538). Concs: A conc resulting in saturated vapour atmosphere (47465 ppm) caused cytotoxicity in strains TA1535 and TA1537. For this reason, a conc of 23732 ppm (118.7 mg/L) was tested. ± S9	**Negative** No significant increase in the frequency of revertant colonies at 23732 ppm, ± S9. As the highest non-cytotoxic concentration did not cause mutagenicity, no additional concentrations were tested to investigate the concentration-response relationship.	Anonymous 27, 1980
In vitro gene mutation study bacteria OECD TG 471, non-GLP Deviation: only 3 strains tested without metabolic action. Reliability: 2 (according to registration dossier, however reporting deficiencies)	Nitromethane (purity unknown) Strain: *S. typh* (TA98, TA100, TA102) Concs: unspecified, but up to 200 µmol/plate. Only without S9. Vehicle: not specified.	**Negative** Did not induce gene mutations in absence of S9 mix in the three strains. Limitations: unclear whether protocol had been adapted for volatile compounds and, consequently, it remained unknown to which concentrations cells had been exposed.	Dayal *et al*. 1989
In vitro chromosomal aberration study in mammalian cells	Nitromethane (purity unknown) Cell type: CHO cells	**Negative** No cytotoxicity observed at the limit conc.	NTP, 1997

Chinese hamster ovary (CHO) OECD TG 473, non-GLP Reliability: 2 (according to registration dossier)	Concs: 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	Did not induce chromosomal aberration in CHO cells, both ± S9, up to limit concentration of 4980 µg/mL. Limitations: unclear whether protocol had been adapted for volatile compounds and, consequently, it remained unknown to which concentrations cells had been exposed.	
In vitro sister chromatid exchange (SCE) assay in mammalian cells CHO cells OECD TG 479, non-GLP Reliability: 2 (according to registration dossier)	Nitromethane (purity unknown) Cell type: CHO cells Concs: 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 26h: 497, 1655 and 4965 µg/mL ± S9 Vehicle: distilled water	**Negative** No cytotoxicity observed at the limit conc No induction of SCE in CHO cells both ± S9, up to limit concentration of 4965 µg/mL. Limitations: unclear whether protocol had been adapted for volatile compounds and, consequently, it remained unknown to which concentrations cells had been exposed.	NTP, 1997
In vitro micronucleus test in Syrian	Nitromethane (purity unknown)	Negative	Gibson *et al.* 1997

hamster embryo (SHE) cells Non-GLP Reliability: 2 (according to registration dossier)	Cell type: SHE cells Concs: - With DMSO: 0, 5.0, 5.5 and 6.0 µg/mL - With media: 0, 3500, 4000, 5000 (µg/mL) No metabolic activation. Vehicle: DMSO or media	Nitromethane did not induce an increased frequency of micronuclei in SHE cells.	
In vitro cell transformation study in mammalian cells EU Method B.21, non-GLP Reliability: 2 (according to registration dossier)	Nitromethane (purity unknown) Cell type: Syrian hamster embryo (SHE) Concs: 2000, 2500, 3000, 3500, 4000 and 5000 µg/mL -Exposure for 24 h followed by 6-7 d of growth -Exposure for 7 d Vehicle: DMSO	**Positive** Dose-dependent significant increase in the morphological transformation frequency at the two highest concentrations. Note: test responds to different mechanisms, including non-mutagenic mechanisms, so outcome alone does not provide evidence for mutagenicity. May be predictive of carcinogenicity (see carc section).	Kerckeart *et al.* 1996
***In vivo* data - nitromethane**			
In vivo micronucleus assay in normochromatic	Nitromethane (purity unknown)	Negative No increase in the frequency of micronucleated erythrocytes observed in the	NTP 1997

erythrocytes (NCEs) of mice OECD TG 474, non-GLP B6C3F1 mouse, 10/sex Inhalation Reliability: 2 (according to registration dossier)	Concs: 94, 188, 375, 750 and 1500 ppm (=limit dose) Exposure: 6h/d, 5d/week for 13 weeks	peripheral blood of either sex of mouse, up to the limit dose of 1500 ppm. Limitations: unclear whether the substance reached the bone marrow	

RAC conclusion

RAC noted that the metabolism of nitromethane is suggested to lead to the formation of formaldehyde, which has a harmonised classification of Muta. 2 (H341). However, no complete *in vivo* metabolism of nitromethane has been reported. Metabolism of nitromethane by rat liver microsomes resulted in the formation of trace amounts of formaldehyde.

Various uncertainties were identified in the data set. Many of the *in vitro* studies did not indicate whether the protocol had been adapted for volatile compounds, consequently, it is unclear what concentrations cells were exposed to. To add, no *in vitro* data of chromosomal aberration or micronucleus tests were provided. RAC noted that the data are inconclusive to allow characterisation of the complete mutagenic profile of the compound.

Based on the studies reported, including the data on nitroethane and 1-nitropropane, RAC concluded that the evidence for classification of nitromethane for germ cell mutagenicity is inconclusive and therefore no classification is warranted.

Classification proposed by the Agency:

The Agency agrees with RAC's conclusion on the classification. Classification for germ cell mutagenicity is not warranted for nitromethane, based on inconclusive evidence.

Carcinogenicity

Classification agreed by RAC:

The assessment for carcinogenicity was based on the data for nitromethane itself. RAC considered the available studies on nitroethane and 1-nitropropane as uninformative due to low dosing and low animal numbers.

The key studies in RACs assessment were the long term (2-year) inhalation studies in rats and mice, conducted by NTP (1997). These were considered key studies as they examined numerous endpoints across both sexes of two species, met standards of study design and performance, and were peer reviewed by international bodies. Exposure to nitromethane resulted in tumours across both rodent species, in different tissue sites. Nitromethane has also been previously reviewed by groups such as IARC who have denoted it as a Group 2B (possible human carcinogen).

Experimental data

In the 2-year rat study, F344/N rats (50/sex/group) were exposed to nitromethane via inhalation at concentrations up to 375 ppm (equivalent to 0.94 mg/L). Mortality was relatively high across all dose groups in both sexes, but was not dose-related. Body weights were unaffected in males, but a slight increase in body weight was observed within top dose females compared to control animals. No further non-neoplastic effects were reported.

No neoplastic effects were observed in males. Masses on the shoulders and torso were observed in females in the 188 ppm and 375 ppm groups; these were considered to be consistent with mammary gland neoplasms. The incidence of fibroadenoma and combined adenoma, fibroadenoma, or carcinomas, were dose-dependent and statistically significant within the 188 and 375 ppm groups. Tumour incidences for these tumour types were outside the range of the historical control data (HCD) for all treated animals. Carcinomas were outside of the HCD in the low and high dose groups only, however, statistical significance was observed at the top dose of 375 ppm. Carcinomas were reported as appearing earlier in treated groups, compared to controls. All control group incidences were within HCD.

Table 4: Tumour incidence (%) within the mammary gland of F344 female rats observed in NTP (1997), with corresponding historical control data range.

	0 ppm 0 mg/L	94 ppm 0.235 mg/L	188 ppm 0.47 mg/L	375 ppm 0.94 mg/L	Historical control data (%)[a]
Adenoma	2/50 (4%)	0/50 (0%)	0/50 (0%)	2/50 (4%)	(0-4%)
Fibroadenoma	19/50 (38%)	**21/50 (42%)**	**33/50 (66%)****	**36/50 (72%)****	(20-40%)
Carcinoma	2/50 (4%)	**7/50 (14%)**	1/50 (2%)	**11/50 (22%)***	(0-8%)
Adenoma, fibroadenoma, or carcinoma	21/50 (42%)	**25/50 (50%)**	**35/50 (68%)****	**41/50 (82%)****	(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 **$p<0.01$. Incidence exceeding HCD indicated in **bold**.

In the 2-year mouse study, B6C3F1 mice (50/sex/group) were exposed to nitromethane via inhalation at concentrations up to 750 ppm (equivalent to 1.87 mg/L). Mortality was high within both sexes across all dose groups, except the survival rate in females from the 750 ppm group which was marginally higher than other groups. No treatment-related effects were observed on bodyweights. Both sexes reported swelling around the eyes and exophthalmos; these are considered to be coincident with observed harderian gland neoplasms. Other non-neoplastic effects included nasal lesions and nasolacrimal duct inflammation (see STOT-RE section for details).

Harderian gland adenomas, carcinomas, and combined (adenoma and carcinoma) rates were similar throughout the study and at termination. A dose-dependent increase in tumours was observed in both sexes, with statistical significance reached in the 375 and 750 ppm groups for adenomas and combined adenoma or carcinoma incidence. RAC noted that this tissue has no equivalent in humans.

Table 5: Summary of observed harderian gland tumour incidence in mice in NTP (1997), with corresponding historical control data range.

		0 ppm 0 mg/L	188 ppm 0.47 mg/L	375 ppm 0.94 mg/L	750 ppm 1.87 mg/L	Historical control data range (%)
Adenoma	M	**9/50 (18%)**	**10/50 (20%)**	**19/50 (38%)***	**32/50 (65%)****	(2-14%)
	F	5/50 (10%)	7/50 (14%)	**16/50 (32%)****	**19/50 (38%)****	(0-16%)
Carcinoma	M	1/50 (2%)	1/50 (2%)	**6/50 (12%)**	**5/50 (10%)**	(0-4%)
	F	1/50 (2%)	2/50 (4%)	**4/50 (8%)**	**3/50 (6%)**	(0-4%)
Adenoma or carcinoma	M	**10/50 (20%)**	**11/50 (22%)**	**25/50 (50%)****	**37/50 (74%)****	(2-14%)
	F	6/50 (12%)	**9/50 (18%)**	**20/50 (40%)****	**21/50 (42%)****	(0-16%)

[a]: HCD from Battelle Pacific Northwest laboratories, in B6C3F1 mice, 1995; * shows statistical significance with the Fisher exact test $p<0.05$ and **$p<0.01$. Incidence exceeding HCD indicated in **bold**.

Alveolar/bronchiolar adenoma and carcinoma were observed in both sexes of mice. A dose-dependent increase in malignant tumours was observed, with statistical significance reached at 750 ppm in males. The incidence at the top dose was also outside the range of the HCD. The combined incidence of adenomas and carcinomas was statistically significantly increased in females at the top dose; the incidence at this dose was outside the range of the HCD.

Table 6: Summary of observed lung tumour incidence in mice in NTP (1997), with corresponding historical control data range.

		0 ppm 0 mg/L	188 ppm 0.47 mg/L	375 ppm 0.94 mg/L	750 ppm 1.87 mg/L	Historical control data range (%)
Alv./bronc adenoma	M	11/50 (22%)	10/50 (20%)	9/50 (18%)	12/50 (24%)	(6-36%)
	F	3/50 (6%)	3/50 (6%)	2/49 (4%)	**9/50 (18%)**	(0-14%)
Alv./bronc carcinoma	M	2/50 (4%)	3/50 (6%)	3/50 (6%)	**11/50 (22%)****	(0-16%)
	F	0/50 (0%)	3/50 (6%)	**5/49 (10%)****	3/50 (6%)	(0-6%)
Alv./bronc adenoma or carcinoma	M	13/50 (26%)	13/50 (26%)	12/50 (24%)	20/50 (40%)	(10-42%)
	F	3/50 (6%)	6/50 (12%)	6/49 (12%)	**12/50 (24%)****	(0-16%)

[a]: HCD from Battelle Pacific Northwest laboratories, in B6C3F1 mice, 1995; Alv/Bronch = alveolar / bronchiolar; * shows statistical significance with the Fisher exact test $p<0.05$ and **$p<0.01$. Incidence exceeding HCD provided in **bold**.

Liver tumours were observed in females only. However, the incidence of hepatocellular adenomas, carcinomas and combined adenoma or carcinoma did not have dose dependence. Statistical significance was observed in the adenomas and combined adenoma/carcinomas in the 188 ppm and 750 ppm groups. Tumour latency appeared to reduce with dose for adenomas and combined adenoma or carcinomas.

Table 7: Summary of observed liver tumour incidence in female mice in NTP (1997), with corresponding historical control data range.

	0 ppm 0 mg/L	188 ppm 0.47 mg/L	375 ppm 0.94 mg/L	750 ppm 1.87 mg/L	Historical control data range (%)
Hepatocellular adenoma	14/50 (28%)	**25/49 (51%)****	17/49 (35%)	**35/50 (70%)****	(0-40%)
Hepatocellular carcinoma	10/50 (20%)	14/49 (29%)	8/49 (16%)	12/50 (24%)	(2-30%)
Hepatocellular adenoma or carcinoma	19/50 (38%)	**34/49 (69%)****	22/49 (45%)	**40/50 (80%)****	(6-54%)

[a]: HCD from Battelle Pacific Northwest laboratories, in B6C3F1 mice, 1995; * shows statistical significance with the Fisher exact test $p<0.05$ and **$p<0.01$. Incidence exceeding HCD provided in **bold**.

An additional study on nitromethane (Anonymous 34, 1990) in Long-Evans rats utilised concentrations of 0, 100 or 200 ppm (0, 0.25, 0.50 mg/L respectively) to investigate long term toxicity. No treatment-related effects, neoplastic or non-neoplastic, were reported.

RAC conclusion

RAC considered that a causal relationship has been established between nitromethane exposure and an increased incidence of a combination of benign and malignant neoplasms in two species (rats and mice).

Nitromethane was considered to have induced an increased incidence of mammary tumours in female rats, with statistical significance in carcinomas at the highest dose, and an increased incidence of combined benign/malignant tumours at the two highest doses. Tumours in mammary glands were considered to increase in a dose-dependent manner, in the absence of confounding systemic toxicity, and occurring earlier than in control animals.

In mice, nitromethane was considered to induce dose-dependent malignant tumours in the lungs (alveolar/bronchiolar carcinoma) of both sexes. The available HCD suggest these tumours are not common within this strain of mouse (B6C3F1). In addition, an increased incidence of benign tumours in the liver were observed in females. Finally, a dose-dependent, significant increase in Harderian gland tumours were observed in both sexes

of mouse. Although this tissue has no equivalent in humans, the observation of these tumours in rodents is seen as an indication of carcinogenic potential as part of the weight of evidence analysis, especially when reported in association with other tumours (multisite response).RAC considered that the lack of observations from the study by Anonymous 34 (1990) does not reduce the concern for the tumours observed in the NTP (1997) studies, as the NTP studies are considered reliable. In addition, doses within the Anonymous 34 (1990) study were lower than those in the NTP studies.

For potential mechanisms of carcinogenesis, the mechanism by which nitromethane causes cancer is unknown. Nitromethane is structurally related to some other nitro compounds which have been evaluated as possibly carcinogenic- it is hypothesised that reactive radicals may play a key role in carcinogenicity. RAC noted that mechanistic information may increase concern or support read-across for category members. Nitromethane gave negative results in all available tests for mutagenic effects, but the overall evidence is considered inconclusive for mutagenicity.

Although the mechanism by which nitromethane causes cancer is not known, nitromethane was reasonably anticipated to be a human carcinogen by RAC, based on the tumour profile induced in rodents.

RAC concluded that classification as Carc. 1B, H350 (may cause cancer) is therefore warranted.

Classification proposed by the Agency:

The Agency considers that nitromethane has been shown to increase the incidence of mammary tumours in female rats through a statistically significant increase in carcinoma at the highest dose combined with benign and malignant tumours at the two highest doses in the NTP (1997) study. These mammary gland tumours were statistically significantly increased in a dose-dependent manner in rats in the absence of confounding systemic toxicity. Furthermore, it was demonstrated that the observed tumours occur earlier in treated animals than in control animals (decreased latency), a dose-dependent increase in the severity of the lesions was also reported, as a statistically significant number of carcinomas were observed at the highest dose.

The Agency also agrees with RAC that in mice following exposure to nitromethane, the incidence of malignant tumours such as alveolar/bronchiolar carcinoma were increased in the lungs of both sexes (see table 6 above). Furthermore, a statistically significant increased incidence in liver neoplasms (primarily adenomas) in female mice was identified. Taken together, nitromethane exhibits carcinogenic effects in rats and mice (benign and malignant tumours in mammary gland in rats and in liver and lungs in mice). The Agency considers that the neoplasms in the harderian gland are supportive information only as they do not have an equivalent in humans but may serve as an indication of the overall carcinogenic potential of nitromethane.

The Agency also notes the reviews of the following international bodies:

- IARC Carcinogen - Possible human carcinogen (Group 2B) (IARC 2000)
- NTP Carcinogen - Reasonably anticipated to be a human carcinogen (NTP 2021)
- ACGIH (the American Conference of Governmental Industrial Hygienists) Carcinogen – 3A Confirmed Animal (ACGIH 2024)

Overall, the Agency can support the conclusion of RAC that **classification as Carc.1B, H350 (~May cause cancer) is warranted**.

Reproductive toxicity

Classification agreed by RAC:

As RAC rejected the category approach proposed by the DS for read-across among the three category members for the assessment of reproductive toxicity, only the substance-specific data on nitromethane were assessed for classification.

Sexual function and fertility

No guideline studies with detailed investigations into sexual function and fertility with nitromethane were available.

In the 13-week inhalation toxicity studies on rats and mice (NTP, 1997), limited parameters were assessed. F344 rats and B6C3F1 mice (10/sex/group) were exposed to nitromethane vapours up to 1500 ppm (corresponding to 3.74 mg/L). No mortality was observed in rats or mice. At 1500 ppm, a statistically significant decrease in body weight and body weight gain, testis (-7%), epididymis (-12%) and cauda (14%) weights was observed in male rats. Sperm motility was statistically significantly decreased from 750 ppm (1.87 mg/L) and above. Sperm count was unaffected at all doses.

RAC noted that from 375 ppm (0.938 mg/L) and above, Ht and Hb values were decreased and RBC count was increased without clear concentration-response relationship, in parallel with an increased MetHb level (up to 173% at the highest concentration). A potential mechanism of reduced sperm motility may be hypoxia, as a consequence of the microcytic anaemia. No effects on oestrus cycle were observed in female rats.

In the mice, no changes to body weight or body weight gain were observed. Organ weight changes lacked clear dose-response relationship and were not considered treatment related. No effects were observed in male mice on the testis, epididymis, cauda weights, or sperm count. However, sperm motility was statistically significantly decreased at all doses, although without a clear dose-response relationship. In female mice, oestrus cycle length was statistically significantly longer with a clear dose-response relationship: 4 days at 0 ppm, 4.33 days at 375 ppm, 4.5 days at 750 ppm and 4.71 days at 1500 ppm.

RAC considered that classification for category 1A/1B was not appropriate due to a lack of human data, and the animal data did not provide clear evidence for adverse effects on sexual function and fertility. No guideline reproductive toxicity study with dedicated investigations of sexual function and fertility for nitromethane were available. In the available 13-week studies, decreases in testis, epididymis, cauda weights and sperm motility were observed in parallel with lower body weight in rats. However, these effects were in the presence of systemic toxicity, with RAC citing hindlimb paralysis and haematological findings in all animals exposed to these doses. For mice, no treatment-related effects on sexual organ weights were observed. A statistically significant decrease in sperm motility from 375 ppm was observed in males, and significantly longer oestrus cycle length in females. RAC considered that the observed effects do not provide sufficient evidence for classification under sexual function and fertility, although RAC did consider that they raise a concern for potential effects. However, due to a lack of dedicated studies, RAC concluded that it remains inconclusive whether nitromethane has hazardous properties on sexual function and fertility.

RAC concluded that no classification for sexual function and fertility is warranted due to inconclusive data.

Adverse effects on development

In a guideline (OECD TG 414, GLP) prenatal developmental toxicity study (Anonymous, 2017), Wistar rats (24 pregnant females/group) were exposed to nitromethane vapours at concentrations of 0, 300, 600 and 1200 ppm (equivalent to: 0, 0.75, 1.50 and 3 mg/L). Exposure occurred for 6h/d from GD 6 to 20.

Maternal body weights were statistically significantly decreased at days 18 (-5%) and 21 (termination, -13%) at 1200 ppm, when not corrected for gravid uterine weight. Food consumption was statistically significantly reduced in the 1200 ppm dose group during GD 6-9 (-16%) and GD 18-21 (-11%). Statistical significance was not reached on continuous days or with a clear dose-response relationship. Gravid uterus weight, relative ovarian weight, relative liver weight and absolute and relative kidney weights were statistically significantly reduced. When correcting body weight at termination for gravid uterus weight, no statistically significant differences were observed between groups.

Several parameters were affected at the top dose. A statistically significant increase in mean post-implantation loss (6.9, vs 0.3 in control) and the percentage of late resorptions (53.8%, vs 2.2% in control) was observed. Mean foetal number and foetal body weights were statistically significantly reduced compared to controls. The mean number of live foetuses/animal were statistically significantly decreased at 5.7 compared to 11.9 in controls.

External malformations and variations showed increased incidences at 1200 ppm, including the number of foetuses with malformations per litter (8.4% per litter, compared to

1.2% in controls) and the number of variations per litter (94.4% per litter, statistically significant compared to 68.9% in controls). Subcutaneous oedema was listed as an external malformation and observed in one foetus from the high dose group. For variations, a statistically significant increase in the number of pale foetuses was observed at the top dose (+76.5%, 13/17 litters) compared to controls. This may be a result of increased MetHb levels and anaemia, although haematological parameters were not monitored in dams or foetuses.

The number of foetuses with skeletal malformations was increased at 1200 ppm (10, vs 2 in control). One control animal was observed with an absent and branched rib. At 1200 ppm, sternebrae malformations (split and fused) were observed in 9 animals. The number of skeletal variations was high across all groups, with 97.1, 99.1, 95.8 and 100% of animals reported with variations (corresponding with 0, 300, 600, 1200 ppm respectively). Variations included unossified digits, incomplete ossification of the pubis and incomplete ossification of the metatarsals, where statistical significance was reached at 1200 ppm. Wavy ribs showed a statistically significant increase from 600 ppm.

RAC considered that classification under category 1A was inappropriate, based on a lack of human data. Category 1B can be considered on the basis of animal data providing clear evidence on developmental effects without maternal toxicity, or, if occurring together with other toxic effects, effects on development are not considered secondary non-specific consequences of the other effects. The presence of maternal toxicity shall not be used to negate embryo-foetal effects, unless these effects are clearly demonstrated to be secondary, non-specific effects. This is considered the case when effects on the offspring are significant, such as structural malformations.

In the prenatal developmental toxicity study, non-corrected body weight, body weight gain and food consumption were reduced in animals at 1200 ppm. However, this was without clear dose-response relationship and continuous statistical significance. The corrected maternal body weight was not significantly different in treatment groups, therefore, the observed significant reduction in gravid uterus weight is considered due to post-implantation loss and reduced pup body weight. The mean number of live foetuses, foetal body weights and increased incidences of external and skeletal malformations and variations were observed at 1200 ppm. RAC considered that these observed effects provide clear evidence of adverse developmental effects, not co-occurring with failure to thrive, severe inanition, prostration or death, and are not considered to be secondary non-specific consequences of maternal toxicity.

Overall, RAC concluded that classification for developmental toxicity category 1B is warranted.

Effects on or via lactation

No data were available for the assessment of effects on or via lactation; RAC concluded that no classification was warranted due to a lack of data.

Classification proposed by the Agency:

Sexual function and fertility

The Agency agrees with RAC's conclusion on the classification. Classification for sexual function and fertility is not warranted for nitromethane, due to inconclusive data.

Adverse effects on development

The Agency agrees with RAC's conclusion on the classification.

At the top dose of 1200ppm, a statistically significant increase in post-implantation loss (50.3% versus 2.2% in control), a statistically significant decrease in mean number of live foetuses (5.7 versus 11.9 in control) and a lower bodyweight (24% and 21% in females and males, respectively versus control) were reported. With regards to malformations and variations, the number of foetuses with skeletal malformations increased at 1200 ppm, with statistically significant increases in the number of sternebrae malformations (split and fused). These effects are considered relevant for classification under adverse effects on development.

Overall, the Agency agrees with RAC that the effects described provide clear evidence of adverse developmental effects in animals that do not occur as a result of secondary non-specific consequences of maternal toxicity.

Therefore**, the Agency concludes that classification for developmental toxicity Category 1B; H360D (May damage the unborn child) is warranted.**

Effects on or via lactation

The Agency agrees with RAC's conclusion on the classification. Classification for lactation is not warranted for nitromethane due to a lack of data.

Aspiration hazard

Not assessed in the CLH report or RAC opinion.

Environmental hazards

Hazardous to the aquatic environment

Not assessed in the CLH report or RAC opinion

Other hazards

Hazardous to the ozone layer

Not assessed in the CLH report or RAC opinion

Overall conclusion

The Agency has evaluated the RAC Opinion, its rationale and any additional scientific evidence that may have been made available to HSE against the criteria for classification and labelling in the GB CLP Regulation and technical guidance.

The Agency technical report **agrees** with the classification proposed by RAC for the following hazards:

Acute Tox. 4; H302 (Harmful if swallowed) with an ATE of 1450 mg/kg bw

Acute Tox. 4; H332 (Harmful if inhaled) with an ATE of 11 mg/L (vapour)

STOT RE 2; H373 (May cause damage to the respiratory tract and nervous system through prolonged or repeated exposure)

Repr. 1B; H360D (May damage the unborn child)

Carc. 1B; H350 (May cause cancer)

Overall, the conclusion is to **agree** with the RAC opinion.

References

ECHA (2024) Guidance on the Application of the CLP Criteria, Part 3: Health Hazards. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 5.0, ref: ECHA-24-G-06-EN. Available at https://www.echa.europa.eu/

For all other references, please see the EU CLH report and the EU RAC opinion (available at: https://echa.europa.eu/registry-of-clh-intentions-until-outcome)

CLH (2023) CLH report (including Annexes): Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance Name: nitromethane; Date: 2023; Written by: Belgian Federal Public Service Health, Food Chain Safety and Environment Accessed date: 03/2026

ECHA (2025) Committee for Risk Assessment (RAC) Opinion (including Annexes) proposing harmonised classification and labelling at EU level of nitromethane; Reference CLH-O-0000007480-77-01/F; Date: 30/09/2025, Accessed date: 03/2026

Documents published as part of the EU CLH process: Source: European Chemicals Agency, http://echa.europa.eu/

Glossary of terms used in Agency technical reports

Agency, the	HSE, acting in its capacity as the GB CLP Agency
AR	Applied radioactivity
ATE	Acute toxicity estimate
BCF	Bioconcentration factor
BOD	Biological Oxygen Demand
bw	Body weight
CAR	Competent Authority Report
CAS	Chemical Abstracts Service
CHO	Chinese Hamster Ovary
CI	Confidence interval
CL	Confidence limits
CLH	Harmonised Classification and Labelling
CLP	Classification, labelling and packaging (of substances and mixtures)
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
CV	Coefficient of Variation
d	Day
DAR	Draft Assessment Report
DOC	Dissolved Organic Carbon
DS	Dossier Submitter
DT	Dissipation time OR degradation time (also DissT or DegT where apparent)
DT_{50}	Dissipation half-life OR degradation half-life (hours or days), see also above
dw	Dry weight
ECHA	European Chemicals Agency
ECx	x% effect concentration
EFSA	European Food Safety Authority
E_rCx	x% effect concentration based on growth rate
EU	European Union
GLP	Good Laboratory Practice
h	Hours
Hb	Haemoglobin
Ht	Haematocrit
K_{oc}	Organic carbon-water partition coefficient
K_{ow}	Octanol-water partition coefficient
LC_x	x% lethal effect concentration

MCL	Mandatory Classification and Labelling
MetHb	Methaemoglobin
M-factor	Multiplying factor
MW	Molecular weight
NOEC	No-observed effect concentration
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
QSAR	Quantitative structure-activity relationship
PCV	Packed cell volume
RAC	Risk Assessment Committee
RAR	Renewal Assessment Report
RBC	Red blood cell
RCOM	Response to comments document
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals regulation
SHE	Syrian Hamster embryo
STOT-RE	Specific target organ toxicity – repeated exposure
STOT-SE	Specific target organ toxicity – single exposure
TG	Test Guideline
US EPA	United States Environmental Protection Agency
WBC	White blood cell
wt	Weight
wwt	Wet weight

Further information

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