COMPILED COMMENTS ON CLH CONSULTATION

Comments provided during consultation are made available in the table below as submitted through the web form. Please note that the comments displayed below may have been accompanied by attachments which are listed in this table and included in a zip file if non-confidential. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

ECHA accepts no responsibility or liability for the content of this table.

Last data extracted on 23.04.2024

Substance name: nitromethane
CAS number: 75-52-5
EC number: 200-876-6
Dossier submitter: Belgium

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	United Kingdom	Health and Safety Executive	National Authority	1
Comment received				
The classification assessment for nitromethane, 1-nitropropane and nitroethane relies on read-across for some hazard classes (e.g. carcinogenicity in which the DS relies on two nitromethane studies to propose Carc. 1B for nitroethane and 1-nitropropane). The current read-across justification in the CLH report, which is publicly available, is lacking some considerations laid out in the RAAF. For example, information related to 'AE C.4 Consistency of effects in the data matrix' has not been provided. The DS does refer to a read-across justification document which is within the confidential Annex I of the CLH dossier. We also note the existence of a publication (Garnick et al 2021 - https://doi.org/10.1002/jat.4169) which questions several of the classification proposals from the DS. Therefore, in the interests of transparency, would the DS be able to provide as much of the read-across justification as possible without breaching confidentiality or would RAC be able to provide a further analysis within their opinion?				

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2024	Germany		MemberState	2
Comment received				
The CLH proposal for nitromethane was provided along with two further CLH proposals for other short chained nitroparaffins, namely nitroethane and 1-nitropropane. In these three dossiers, read-across between all three substances was used, except for the endpoint acute toxicity in which substance-specific data were used for the respective substances. The proposed classification for the substances is supported. Please review the information regarding the partition coefficient n-octanol/water for nitromethane in table 6:				

Nitromethane: LogKow = 0.574 at 21.8 °C, pH 7 given in table 6 and -0.33 in table 7 Supposing that the data in tables 6 are correct, the LogKow for nitromethane is 0.574. Please check and correct if necessary.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	3
Comment received				
The read-across between nitromethane, nitroethane and 1-nitropropane is very well explained and justified for all the endpoints. FR noted that the substances are part of the GMT 316. The substance 2-nitroproprane, also part of the GMT 316, is mentioned in the CLH report as “can reasonably be expected to be human carcinogens”. 2-nitropropane has a harmonized classification as Carc. 1B, This information may have been used to support the classification proposal as Carc.1B for the 3 nitroalkanes. In the CLH report, the DS noted that “the metabolism of nitromethane leads to the formation of formaldehyde which has a harmonised classification as Muta. 2, H341” (page 50) and as “supporting evidence that the metabolism of nitromethane leads to the formation of formaldehyde which has a harmonised classification as Carc. 1B” (page 75). FR asks to clarify this statement “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.” (Read-across justification between nitromethane, nitroethane and 1-nitropropane, page 11) which is not in accordance with what is stated above.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2024	France	ANGUS Chemie GmbH	Company-Importer	4
Comment received				
All comments in each specific section below, are submitted on behalf of: - Advancion Corporation, the largest and only fully integrated global manufacturer of the substance, located in USA, - and ANGUS Chemie GmbH, its German branch, the EU importer and REACH lead registrant of the substance. We would like to stress as an intro, that given the Joint submission full dossier tonnage band (1-10t), there are no data requirements for 1) repeat-dose toxicity data, 2) reproductive/developmental toxicity data, 3) carcinogenicity data. ECHA note – An attachment was submitted with the comment above. Refer to public attachment NM PUBLIC attachments.zip				

HEALTH HAZARDS – Acute toxicity

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2024	Germany		MemberState	5
Comment received				
For acute toxicity (oral and inhalation), conclusive data for each of the individual substances is available and thus classification proposals for acute toxicity were based on the data on				

the particular substances. Acute Tox. 4 (oral) is proposed for all three substances. For the inhalation route, Acute Tox. 3 is proposed for nitromethane and 1-nitropropane and Acute Tox. 4 for nitroethane. ATE values are proposed based on the data for the individual substances.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	6
Comment received				
FR agrees with the classification of nitromethane for acute oral toxicity as Acute Tox. 4; H302 (Harmful if swallowed) based on an ATE of 1450 mg/kg bw (> 300 but ≤ 2000 mg/kg bw). About acute toxicity via dermal route, it is mentioned “Hazard class not evaluated in this CLH dossier”. FR is wondering why read-across was not performed with 1-nitropropane for this endpoint? FR agrees with the classification of nitromethane for acute inhalation toxicity as Acute Tox. 3, H331 based on an ATE of 5.50 mg/L (2.0 ≤ ATE ≤ 10.0 mg/L.)				

HEALTH HAZARDS – Germ cell mutagenicity

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2024	Germany		MemberState	7
Comment received				
Based on the available in vitro and in vivo data for all three substances, which is considered inconclusive, classification for this endpoint is not proposed.				

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	8
Comment received				
FR agrees that data are inconclusive for the classification of nitromethane for germ cell mutagenicity.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2024	France	ANGUS Chemie GmbH	Company-Importer	9
Comment received				
Acronyms: DS = dossier submitter (Belgium), 1-NP = 1-nitropropane (CAS 108-03-2), NE = nitroethane (CAS 79-24-3), NM = nitromethane (CAS 75-52-5). The DS stated in the discussion of carcinogenicity that NM “was not found to be genotoxic” (CLH Report for NM, 2023: p. 76). A WoE evaluation of NM genotoxicity performed by Garnick et al. (2021 -ATTACHED) concluded the compound was not genotoxic. ECHA note – An attachment was submitted with the comment above. Refer to public attachment NM PUBLIC attachments.zip				

HEALTH HAZARDS – Carcinogenicity

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2024	Germany		MemberState	10

Comment received
The proposal for classification as Carc. 1B, H350 is supported. Please note that the IARC evaluation leading to classification for carcinogenicity in IARC Category 2B is from the year 2000 and included the studies that are evaluated in the CLH report. Classification is based on a 2-year inhalation study using nitromethane in rats and mice performed by the NTP; the available studies for nitroethane and 1-nitropropane show limitations in the study design. Nitromethane induced increased incidences of mammary gland fibroadenomas and carcinomas in female rats. There was no evidence of carcinogenic activity in male rats. In mice an increased incidence in alveolar/bronchiolar adenomas and carcinomas as well as harderian gland adenomas and carcinomas was observed in both sexes. Furthermore, a statistically significantly 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). Neoplasms in the harderian gland are considered as supportive information as they do not have an equivalent in humans. Overall, classification as Carc. 1B is proposed for all three substances based on read across.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	11
Comment received				
FR agrees with the classification of nitromethane as Carc. 1B, (H350 may cause cancer) based on the formation of multiple tumours in two species (benign and malignant tumours in mammary gland of female rat, in liver of female mice and alveolar/bronchiolar in both sexes of mice). About the lung tumours, olfactory epithelium degeneration was reported. FR suggests adding the results of the OECD TG 422 study in the section “10.9.1 Short summary and overall relevance of the provided information on carcinogenicity” about the nasal tissue degeneration as supporting evidence of the possible mode of action.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2024	France	ANGUS Chemie GmbH	Company-Importer	12
Comment received				
Acronyms: DS = dossier submitter (Belgium), 1-NP = 1-nitropropane (CAS 108-03-2), NE = nitroethane (CAS 79-24-3), NM = nitromethane (CAS 75-52-5). We disagree with DS proposal for Carc. 1B (CLH Report Chapter 10.9, pp. 3, 75). We propose no classification based on the below key arguments. Each are further detailed in below paragraphs and fully discussed in Maier 2024 review (ATTACHED, chapter 2): •The mammary tumors observed in the NTP (1997) NM rat carcinogenicity study do not demonstrate a carcinogenic potential. • The NTP (1997) NM mouse carcinogenicity study shows effects secondary to local toxicity that would not be relevant to the range of human exposures. • WoE assessment does not suggest a genotoxic potential for NM.				

Details:
The DS used weight of evidence (WoE), relying primarily on the available data for NM in the proposed classification. However, the available NM data have limitations that preclude their results from being considered in the WoE classification.

Maier 2024, chapter 2.1: THE MAMMARY TUMORS OBSERVED IN THE NTP (1997) NM RAT CARCINOGENICITY STUDY DO NOT DEMONSTRATE A CARCINOGENIC POTENTIAL
Mammary tumors in rats: incidence stayed within the range of historical controls (Garnick et al. 2021 ATTACHED: p. 5-6), meaning that the observed effect was reflecting biological variability rather than NM exposure. In addition, the high background rate of tumors within the F344 strain led NTP to phase out the use of this strain in 2-year chronic toxicity and carcinogenicity studies, beginning in 2006 (Garnick et al. 2021: p. 5). To further the point that mammary tumors in F344 rats in the NTP (1997) study are of limited value in a WoE approach, Griffin et al. (1996), cited as “Anonymous 34, 1990” in the CLH Report, observed no treatment-related tumors from exposure to NM in Long-Evans rats at a comparable exposure levels (200 ppm in Griffin et al. 1996; 180 ppm in NTP 1997) and at comparable dosing regimens.
-
Maier 2024, chapter 2.2: THE NTP (1997) NM MOUSE CARCINOGENICITY STUDY SHOWS EFFECTS SECONDARY TO LOCAL TOXICITY THAT ARE NOT RELEVANT TO THE RANGE OF HUMAN EXPOSURES
The findings in the evaluation of NM carcinogenicity in B6C3F1 mice performed by NTP (1997) are consistent with formaldehyde-related toxicity. Mouse tumours: Harderian tumors have limited relevance for human health (CLH Report for 1-NP, 2023: p. 68, 74) and liver tumors stayed within the historical control range (CLH Report for 1-NP, 2023: p. 74). These do not suggest a carcinogenic potential. Regarding lung tumors, increases were observed at the high-dose, above the historical control range (CLH Report for 1-NP, 2023: p. 63–64). However, the high concentration in the study (750 parts per million [ppm]) was associated with respiratory tract non-neoplastic effects, suggesting that pulmonary tumors are secondary to cytotoxicity. Formaldehyde is a respiratory tract tumorigen at high concentrations by this mechanism, and has a CLP harmonized classification as Carcinogen 1B. These tumours are therefore consistent with formation of formaldehyde (NM's metabolite) . The lung tumors observed at high doses in the NTP (1997) mouse study reflect a general response to significant cytotoxic insult. In addition, as per CLP criteria, for a classification of Category 1B, evidence is needed from “(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” (CLH Report for NE, 2023: p. 73). These conditions are not met because when considering NM data, human-relevant cancers are limited to one species (mouse) in one study (NTP 1997) and one organ (lungs). Thus, the available NM studies are not sufficiently informative for cancer classification.

Maier 2024, chapter 2.3: WOE ASSESMENT DOES NOT SUGGEST A GENOTOXIC POTENTIAL FOR NM
Our conclusion that NM is not classifiable as a carcinogen is further supported by evidence of the non-genotoxicity of NM. The CLH Report on NM states that the “data are inconclusive for germ cell mutagenicity” (CLH Report for NM, 2023: p. 51). The DS stated in the discussion of carcinogenicity that NM “was not found to be genotoxic” (CLH Report for NM, 2023: p. 76). A WoE evaluation of NM genotoxicity performed by Garnick et al. (2023) concluded the compound was not genotoxic.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment NM PUBLIC attachments.zip

HEALTH HAZARDS – Reproductive toxicity

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2024	Germany		MemberState	13

Comment received

Overall, classification as Repr. 1B, H360Df is proposed for all three substances. It is based on the following data:

Sexual function and fertility: Classification is based on an overall weight of evidence approach from all three substances. The available data is limited to OECD TG 413 studies using nitromethane or nitroethane showing spermatotoxic effects in rats and mice as well as a combined screening study (OECD TG 422) using 1-nitropropane in which two females of the mid- and high-dose group failed to become pregnant.

Overall, the available data showed several slight effects on fertility parameters which could be evidence of adverse effects and thus may suggest a classification in category 2:
• Reduced sperm motility in mice and rats from 375 ppm:
The effect is dose-dependent and shows statistical significance and should therefore be considered treatment-related. However, the functional relevance of this finding remains unclear, as it was determined in 13-week studies and not in reproductive toxicity studies.

• Moderate increase in relative testicular weight in mice and rats from 100 ppm:
The moderate increase in relative testicular weight occurred in both species, but was mostly limited to high doses of 350 ppm or more. Only in the combined repeated dose toxicity with reproductive/developmental screening toxicity study with nitropropane a significant increase occurred already at the highest dose of 100 ppm, but without a clear dose-response relationship. It should be discussed whether a moderate increase in relative testicular weight should be considered as adverse even under the influence of effects on body weight and without corresponding histopathological findings.

• Prolonged oestrus cycle from 375 ppm:
The effect on oestrus cycle length that occurred in a 13-week study with nitromethane shows statistical significance and a clear dose-response relationship, and is therefore considered treatment-related even without the availability of HCD. It is unclear whether an elongation from 4.0 to 4.7 days is to be classified as an adverse effect.

Based on the observed effects for nitromethane, nitroethane and nitropropane, the classification proposal as Repr. 2, H361f is comprehensible in principle. However, it is necessary to determine whether a marked systemic toxicity was present, which would have to be taken into account for classification purposes. It is noteworthy that the majority of findings occurred at doses of 350 ppm or higher and that animals of both species and sexes showed significantly elevated methaemoglobin levels after exposure to these doses, which persisted for hours after the end of exposure. Since the animals exhibited partly drastically elevated methaemoglobin levels (up to over 70 %) for a large part of the study duration and hypoxic conditions are associated with effects on spermatogenesis it may be suspected that the observed effects on reproductive parameters are secondary to a primary haematotoxicity of the three substances. However, it remains unclear why, despite the high methaemoglobin levels, apparently no behavioural abnormalities were observed.

Development: Classification for developmental toxicity is based on a prenatal developmental toxicity study in rats using nitromethane. For nitroethane and 1-nitropropane, OECD TG 414 studies are not available. Effects identified in the available study include significantly higher post implantation loss and late resorptions, significantly reduced pup body weight, significant increase in the number of pale foetuses (consistent with haematological effects),

and in the number of foetuses with malformations and variations (malformed sternebra, wavy ribs and incomplete ossification of metatarsal).

The observed, in some cases drastic effects on development are also largely limited to high-doses (here: 1200 ppm), at which high methaemoglobin levels are to be expected. In particular, a marked increase in post-implantation loss and late resorption, statistically significant reduced foetal weights and an increase in malformations (malformed sternebra in 9/17 animals, 0 in control animals) should be highlighted here.

Apart from these effects at high doses, a reduced litter size was observed for nitropropane already at the highest dose of 100 ppm. Although the reported litter size is outside the HCD, it shows neither a dose-response relationship nor statistical significance. In addition, due to a lack of individual animal data, it is unclear whether the reduced litter size could be attributed to effects on fertility or development.

Here, as under sexual function and fertility above, a central question for the classification for reproductive toxicity is whether or not the observed methaemoglobin levels are to be assessed as marked systemic.

Lactation: It is agreed that data on lactation is inconclusive for classification.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	14
Comment received				
FR agrees with the classification of nitromethane as Repr. 2, H361f. FR suggests in the “comparison to CLP criteria section” insisting on the fact that fertility is only assessed in the OECD TG 422 study on 1-nitropropane, at low doses, thus suggesting potential effects of nitromethane if animals were mated. FR suggests to insist on the lack of sperm parameters assessments in several studies. FR agrees with the classification of nitromethane as Repr. 1B, H360D. In section 7 PHYSICOCHEMICAL PROPERTIES, Read-across justification between nitromethane, nitroethane and 1-nitropropane), FR suggests adding the results of the OECD TG 414 study on 1-nitropropane in addition to results of the OECD TG 422 study on nitromethane to support the classification proposal for developmental toxicity.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2024	France	ANGUS Chemie GmbH	Company-Importer	15
Comment received				
Acronyms: DS = dossier submitter (Belgium), 1-NP = 1-nitropropane (CAS 108-03-2), NE = nitroethane (CAS 79-24-3), NM = nitromethane (CAS 75-52-5). We disagree with DS proposal for Repr. 1B (H360Df, CLH Report pp. 101, 103). We propose no classification based on the below key arguments. Each are further detailed in below paragraphs and fully discussed in Maier 2024 review (ATTACHED, chapter 3): • The sperm effects observed in NM/NE studies are secondary to hypoxia and hence do not require a classification for effects on reproduction. • Available NE data are sufficient to conclude that NE should not be classified as a developmental toxicant. • Available NE data are sufficient to conclude that NE should not be classified as a reproductive toxicant.				

Details:
For development, the DS relied on a prenatal developmental toxicity study performed with nitromethane (Anonymous 36, 2017), where 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." (CLH Report for NM, 2023: p. 103). Concerning fertility, the DS concluded that sperm effects seen in NM subchronic studies warrants a classification of Repr. 2 (H361f) (CLH Report of NM, 2023: p. 5, 103).

Maier 2024, chapter 3.1: THE STUDIES USED TO CLASSIFY NM AS A DEVELOPMENTAL TOXICANT EXCEEDED THE MTD AND ARE NOT RELIABLE FOR CLASSIFICATION
NM data clearly show that hematological effects, including those related to tissue oxygenation, occur at/or concentrations well below developmental effects. As described by Lewis et al. (2024-ATTACHED), maximum doses for a reproductive study need to consider other biological response mechanisms that induce developmental toxicity secondary to toxicity in the dams. One specific mechanism noted by Lewis et al. (2024) is anemia and hypoxia, both of which are known effects of NM at high doses (Garnick et al. 2021-ATTACHED).

Maier 2024, chapter 3.2: THE SPERM EFFECTS OBSERVED IN NM STUDIES ARE SECONDARY TO SYSTEMIC TOXICITY AND HENCE DO NOT REQUIRE A CLASSIFICATION FOR EFFECTS ON REPRODUCTION
It should be emphasized that available data to not provide conclusive evidence to classify NM for reproductive toxicity. The DS focused on sperm effects as the primary basis for the reproductive toxicity classification. One hypothesis is that the sperm effects may be secondary to hypoxia, a known effect of nitrite (Reyes et al. 2012 ATTACHED, as described in Garnick et al. 2021: p. 20). In addition, the observed sperm effects for NM come from repeat-dose toxicity studies without any evaluation of reproductive function and, hence, cannot be used for classification.
Overall, while there is a potential for coincident occurrence of systemic toxicity and effects on sperm for nitroalkanes (a plausible mode of action secondary to hypoxia) in subchronic studies, these data do not meet criteria to classify NM for fertility effects, in absence of evaluation of reproductive function.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment NM PUBLIC attachments.zip

HEALTH HAZARDS – Specific target organ toxicity - repeated exposure

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2024	Germany		MemberState	16
Comment received				
The proposed classification is supported regarding STOT RE 2, H373 (blood, nervous system and respiratory tract). Studies investigating effects on the respiratory tract, blood and nervous system are available on each individual substance and these show consistent effects at comparable doses. Overall, classification as STOT RE 2, H372 (respiratory tract, blood and nervous system) is proposed for nitromethane, nitroethane and 1-nitropropane.				

The following effects were described in subacute and subchronic studies:
Respiratory tract: Degeneration in the olfactory epithelium was reported in subacute and subchronic studies.

Nervous system: Reduced brain weights in a 28-day study on 1-nitropropane; sciatic nerve and spinal cord degeneration reported in a 90 day-study with nitromethane. In addition, severe axonal neuropathy in two workers was reported after inhalation of nitromethane.

Blood: Anaemia was characterised by decreases in haematocrit values and haemoglobin concentrations, a higher clotting time and effects on methaemoglobin in subacute and subchronic studies with 1-nitropropane and nitromethane.

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	17
Comment received				
FR agrees with the classification of nitromethane as STOT RE Cat. 2; H373 (May cause damage to organs through prolonged or repeated exposure) (blood, respiratory tract and nervous system) based on the degeneration of the olfactive epithelium, hematological effects and nervous system effects observed in nitromethane studies and by read-across analysis with nitroethane and with 1-nitropropane.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2024	France	ANGUS Chemie GmbH	Company-Importer	18
Comment received				
Given the Joint submission full dossier tonnage band (1-10t), there are no data requirements for repeat-dose toxicity. STOT RE classification conclusions for NM shall only be based on NM data, and these do not evidence irreversible severe effects. On this basis, we disagree with the proposed classification as STOT RE 2 and we propose no classification for repeat-dose toxicity. ECHA note – An attachment was submitted with the comment above. Refer to public attachment NM PUBLIC attachments.zip				

PUBLIC ATTACHMENTS
1. NM PUBLIC attachments.zip [Please refer to comment No. 4, 9, 12, 15, 18]