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.

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Last data extracted on 23.04.2024

Substance name: 1-nitropropane
CAS number: 108-03-2
EC number: 203-544-9
Dossier submitter: Belgium

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2024	Germany		MemberState	1
Comment received				
The CLH dossier on the substance nitropropane has been reviewed with regard to its formal classification and labeling proposal. It is proposed to complete the classification as Acute Tox. 3, H331. From the proposed classification as "Acute Tox. 3, H331" results in accordance with Annex I Part 3 Chapter 3.1 Table 3.1.3 of Regulation (EC) No. 1272/2008(CLP), labeling with the hazard pictogram "GHS06" (skull and crossbones) is mandatory. The hazard pictogram "GHS06" must therefore be added to the CLH dossier. Since the assignment of the hazard pictogram "GHS06" is absolutely necessary the hazard pictogram "GHS07" (exclamation mark) does not appear on the label. The hazard pictogram must be deleted. In the CLH dossier section 2.1 Proposed harmonized classification and labelling according to the CLP criteria, Table 4: - in row "Dossier submitters proposal" and column "Labelling/Pictogram, Signal Word Code(s)" under "Retain" GHS07 should be deleted and GHS06 should be added under "Add"; - in row "Resulting Annex VI entry if agreed by RAC and COM" and column ""Labelling/ Pictogram, Signal Word Code(s)" GHS07 should be deleted and GHS06 be added; - in cell "Specific Conc. Limits, M-factors" the expression "and ATEs" should be added; - in column "Specific Conc. Limits, M-factors" the inhalation ATE value should be mentioned as follows: “inhalation: ATE = 5.50 mg/L (vapors)”.				

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	United Kingdom	Health and Safety Executive	National Authority	2
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
17.04.2024	France	ANGUS Chemie GmbH	Company-Importer	3

Comment received

All comments in each specific section below, are submitted on behalf of:
- Advancion Corporation, the sole worldwide commercial manufacturer of 1NP, 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 tonnage band (10-100t), there are no data requirements for 1) any repeat-dose/reproductive/developmental toxicity data beyond the available OECD 422 study, 2) any carcinogenity study. Therefore, there is no regulatory basis to artificially expand this dataset by using inappropriate read-across to other nitroalkanes with a different metabolism (Nitromethane), as was done in the CLH report, disregarding RAAF guidelines published by ECHA.

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

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	4

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 13) which is not in accordance with what is stated above.

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 1-nitroproprane for acute oral toxicity as Acute Tox. 4; H302 (Harmful if swallowed) based on an oral LD50 of 506 mg/kg bw (> 300 but ≤ 2000 mg/kg bw). FR agrees that acute toxicity via dermal route may not be warranted. FR agrees with the classification of 1-nitroproprane for acute inhalation toxicity as Acute Tox. 3; H331 (Toxic if inhaled) based on an ATE of 5.51 mg/L after a 4-hour exposure (> 2.0 but ≤ 10 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
17.04.2024	France	ANGUS Chemie GmbH	Company-Importer	8
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 CLH report on 1-NP states that the "data are inconclusive for germ cell mutagenicity" (CLH Report for 1-NP, 2023: p. 50). However, a WoE evaluation shows that 1-NP is negative. The evidence supporting this assertion has been integrated in Garnick et al. (2021 - ATTACHED), in which the authors concluded that 1-NP is not mutagenic according to numerous well-documented bacterial mutagenicity assays (p. 15). 1-NP was also negative in mutagenicity and micronuclei testing in mammalian liver cells (Roscher et al. 1990 ATTACHED); the positive response in one assay in lung cells found by Roscher et al was attributed to the presence of genotoxic contaminant 2-nitropropane in the test material and lack of adequate controls (CLH Report for 1-NP, 2023; Garnick et al. 2021). In vivo				

micronucleus assays in Sprague-Dawley rats have yielded negative results (Garnick et al. 2021). Further, mechanistic data provide no support for direct DNA interaction under physiological conditions (Garnick et al. 2021).

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

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	9
Comment received				
FR agrees that data are inconclusive for the classification of 1-nitropropane for germ cell mutagenicity.				

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
17.04.2024	France	ANGUS Chemie GmbH	Company-Importer	11
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 read-across from NM used by DS to classify 1-NP as Carc. 1B does not follow Read-Across Assessment Framework (RAAF) principles and is scientifically invalid.
• The NM studies used for read-across do not provide conclusive evidence of the need for a carcinogenicity classification.
• Available data for 1-NP and NE are sufficient to conclude that 1-NP should not be classified as a carcinogen.
• Genotoxicity findings do not suggest a genotoxic potential for 1-NP.

Details:
The DS stated, “The classification proposal for carcinogenicity of nitroethane and nitropropane is fully based on read-across from nitromethane because the available studies on nitroethane and 1-nitropropane are uninformative due to too low dosing and too low animal number. Thus, the key studies for the assessment of carcinogenicity are the 2-year studies in mice and rats on nitromethane (NTP, 1997)” (CLH Report for 1-NP, 2023: p. 74). This approach is invalid for the reasons detailed below. In addition, no carcinogenicity studies are required for 1-NP given the Joint submission tonnage band (10-100t)so there is no reason to try expand the dataset by resorting to irrelevant read-across.

Maier 2024, chapter 2.1.1: The DS Did Not Follow ECHA RAAF in Its Read-Across Approach:
Common breakdown products is a measure of structural similarity for a read-across (ECHA RAAF 2017: p. 6). The DS provided no consideration of the differential breakdown products of 1-NP or NM when utilizing NM carcinogenicity data to classify 1-NP.
As described in Garnick et al. (2021 ATTACHED), denitrification of the primary nitroalkanes, including 1-NP, results in the formation of nitrite (CAS number: 14797-65-0) and an aldehyde with corresponding chain length (p. 19). For 1-NP, NE, and NM, these aldehyde metabolites are propionaldehyde (CAS number: 123-38-6), acetaldehyde (CAS number: 75-07-0), and formaldehyde (CAS number: 50-00-0), respectively.
The DS did not provide any hypothesis or rationale in performing its read-across as to how NM, NE, and 1-NP would exhibit the same properties in terms of carcinogenicity in spite of the differences in metabolites. The fact that the DS failed to consider that formaldehyde is not a metabolite or breakdown product of 1-NP shows that the DS’s read-across disregards RAAF principles published by ECHA.

- Maier 2024, chapter 2.1.2 NM-Specific Metabolite Formaldehyde Makes Read-Across to 1-NP Scientifically Invalid
A comparison of the current harmonized classification of the two aldehyde metabolites is:
1-NP metabolises into Propionaldehyde (CAS: 123-38-6): Not classified for Carc / Not classified for Muta
NM metabolises into Formaldehyde (CAS: 50-00-0): harmonized classification as Carc. 1B / Muta. 2
Using NM as a read-across source to 1-NP that does not metabolize into formaldehyde leads to overconservative conclusions because of the specific genotoxic and carcinogenic potential of formaldehyde. No scientifically sound conclusion about carcinogenicity classification can be derived when using such a scientifically irrelevant read-across approach.

Maier 2024, chapter 2.2 THE NM STUDIES USED FOR READ-ACROSS DO NOT PROVIDE CONCLUSIVE EVIDENCE OF THE NEED FOR A CARCINOGENICITY CLASSIFICATION FOR NM
Even if NM studies were considered (in disregard of RAAF and toxicological principles as explained above), it should be emphasized that there is no conclusive evidence to classify NM as a carcinogen.
- Maier 2024, chapter 2.2.1 The NM Rat Carcinogenicity Study Used for Read-Across to 1-NP Does Not Demonstrate a Carcinogenic Potential
Mammary tumors in rats: incidence stayed within the range of historical controls (Garnick et

al. 2021: 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).
- Maier 2024, chapter 2.2.2 The NM Mouse Carcinogenicity Study Used for Read-Across to 1-NP Shows Effects Secondary to Local Toxicity and Formation of NM-Specific Metabolite Formaldehyde
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) and stress the irrelevance of using NM data for read-across to 1-NP.
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 1-NP, 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).

Maier 2024, chapter 2.3 AVAILABLE DATA FOR 1-NP AND NE ARE SUFFICIENT TO CONCLUDE THAT 1-NP SHOULD NOT BE CLASSIFIED AS A CARCINOGEN
The DS inappropriately dismissed a relevant inhalation study for 1-NP, Griffin et al. (1982 ATTACHED), that found no significant dose-related increase in tumors in male and female rats (10 rats/sex/group) exposed to 1-NP at 0 or 100 ppm. We consider that Griffin et al. (1982 ATTACHED) is an informative negative carcinogenicity study. The exposure duration was 21.5 months, which is near the standard length for animal cancer studies of 24 months. Further, 100 ppm is much greater than human-relevant doses and which is the maximum inhalation dose determined by Carney et al. (2004) based on a range-finding study. In addition, an oral carcinogenicity study by Hadidian et al. (1968) was also negative. Therefore, 1-NP shows no evidence of carcinogenicity.
In addition to 1-NP data showing no need for 1-NP cancer classification, a supportive argument could be read-across to available NE data. Indeed, this read-across seems more appropriate than read-across from NM, given the similarities in structure (close molecular weight) and metabolism end products between 1-NP and NE as opposed to NM (as described in Garnick et al. 2021). The available carcinogenicity study for NE (Griffin et al. 1988 ATTACHED) was a full 2-year study, and therefore was of sufficient duration to assess carcinogenicity. Further, the study utilized sufficiently high doses (up to 200 ppm, which caused significant body weight decreases = reached the maximum tolerable dose as suggested by OECD 451) and evaluated number of rats approaching OECD 451 guidelines at 40/sex/group. As such, the NE Griffin et al. (1998) study is more representative of an OECD 453 study than those available for 1-NP, and further, found no significant dose-related increase in tumors in male and female rats.
Taken together, when the negative carcinogenicity data for 1-NP is supplemented with the negative carcinogenicity data for NE, the WoE conclusion is that there is no evidence of 1-NP carcinogenicity (ECHA 2011; WHO 2021).

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

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	12
Comment received				
FR agrees with the classification of 1-nitropropane as Carc. 1B, (H350 may cause cancer) based on the read across from nitromethane studies showing 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.				

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
17.04.2024	France	ANGUS Chemie GmbH	Company-Importer	14

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):
• Read-across from NM used to classify 1-NP does not follow RAAF principles and is scientifically invalid.
• The sperm effects observed in NM/NE studies are secondary to hypoxia and hence do not require a classification for effects on reproduction.
• Available 1-NP data are sufficient to conclude that 1-NP should not be classified as a developmental toxicant.
• Available 1-NP data are sufficient to conclude that 1-NP should not be classified as a reproductive toxicant.

Details:
For development as for fertility, the DS approach was essentially based on read-across from NM and to a lesser extent, NE (CLH Report for 1-NP, p. 101), due to limitations in an OECD 422 study on 1-NP and absence of higher-tier reproduction studies. It should first be noted that no higher-tier studies are required for 1-NP given the Joint submission tonnage band (10-100t) so there is no reason to try expand the dataset by resorting to irrelevant read-across.

Maier 2024, chapter 3.1 READ-ACROSS FROM NM USED TO CLASSIFY 1-NP AS REPR. 2 DOES NOT FOLLOW RAAF PRINCIPLES AND IS SCIENTIFICALLY INVALID
Read-across from NM to 1-NP is not appropriate for several reasons. First, both form different aldehydes as discussed under CARCINOGENICITY, and this was not discussed by the DS, making the read-across incompliant to RAAF. Second, although NM, NE, and 1-NP could all have a common nitrite metabolite, it is not known that said metabolite is the underlying cause of the observed sperm effects for NM and NE. Furthermore, there are quantitative differences in nitrite formation and elimination between NM, NE, and 1-NP. Lai et al. (1982 ATTACHED) acknowledged the potential discrepancies in nitrite formation among the nitroalkanes, stating, “Nitrite is the major metabolite found in the blood, urine, and various organs after administration of NE, 1-NP, or 2-NP but not NM” (p. 11). Likewise, Sakurai et al. (1980 ATTACHED) noted that NM had a unique binding affinity compared to other nitroalkanes in rat liver microsomes. The DS failed to demonstrate the causal relationship between shared metabolite formation and toxic effect in its read-across from NM to 1-NP. Furthermore, the DS did not take into account kinetic or quantitative differences in rate of nitrite formation. Therefore, the DS’s read-across from NM to 1-NP disregards RAAF principles published by ECHA.

Maier 2024, chapter 3.2 THE SPERM EFFECTS OBSERVED IN NM STUDIES ARE SECONDARY TO HYPOXIA AND HENCE DO NOT REQUIRE A CLASSIFICATION FOR EFFECTS ON REPRODUCTION
Even if these NM/NE studies were to be considered in an overconservative approach disregarding both RAAF and toxicological principles, then it should be emphasized that available data to not provide conclusive evidence to classify these substances 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: ATTACHED p. 20).

Maier 2024, chapter 3.3 AVAILABLE 1-NP DATA ARE SUFFICIENT TO CONCLUDE THAT 1-NP SHOULD NOT BE CLASSIFIED AS A DEVELOPMENTAL TOXICANT
The DS states that “there is no prenatal developmental toxicity study performed on 1-nitropropane and nitroethane” (CLH Report for 1-NP, 2023: p. 101). However, no such study is required at the 1-NP tonnage band. No classification is required for 1-NP based on the absence of observed effects for 1-NP in a guideline compliant OECD 422 study (Carney

et al. 2004, referenced as Anonymous 37 (2003) in the CLH report). This study reported a developmental NOEC of 100 ppm, the highest concentration tested. With regard to the DS's primary criticisms of the Carney et al. (2004) 1-NP study, this study covers premating and post-parturition periods not covered in the NM study used by the DS. Thus, in terms of periods of susceptibility, the 1-NP study is superior. In the NM study, fetal morphological changes occurred at the same concentration as many other effects that were examined in the 1-NP study. In other words, it is not likely that the design of the OECD 422 study for 1-NP would have missed a potential developmental effect related to fetotoxicity that was uniquely identified in the NM study. If anything, this gives further support for the use of the 1-NP study as the primary evidence when determining the classification of 1-NP.
The DS also raised concerns regarding the highest dose tested in the Carney et al. (2004) study of 1-NP. First, a range finding study at 100, 250, 500 ppm found that at the 250 ppm concentration, females had clear onset of clinical signs, and that this dose would have exceeded the MTD (Carney et al. 2004: p. 15). Thus, 100 ppm is likely appropriately close to the onset dose for severe effects. Second, 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. is anemia and hypoxia, both of which are known effects of 1-NP at high doses (Garnick et al. 2021 ATTACHED). Therefore, the highest concentration tested is appropriate in the consideration of avoiding hypoxia. Further, the 100 ppm concentration did induce decreased bodyweight in males and nasal histopathological changes in females suggestive of doses that would induce animal stress. Therefore, the Carney et al. (2004) study is a reliable, negative study showing no developmental concern for 1-NP.

Maier 2024, chapter 3.4 AVAILABLE 1-NP DATA ARE SUFFICIENT TO CONCLUDE THAT 1-NP SHOULD NOT BE CLASSIFIED AS A REPRODUCTIVE TOXICANT
As explained above, the 1-NP OECD 422 study (Carney et al.) included a high enough top-dose showing no concern for fertility as expressed by number of offspring. In addition, no studies on fertility (OECD 416/443) are required at the 1-NP tonnage band.
Carney et al. (2004, referenced as Anonymous 37 2003 in CLH report) on 1-NP found no treatment-related effect on time to mating, gestation length, post-implantation loss, pup survival, or pup-sex ratio in an OECD 422 study in Sprague-Dawley rats exposed to up to 100 ppm. Two females in the mid- and high-dose levels failed to be pregnant, but the CLH report concluded it "cannot be stated if the reduced fertility index can be attributed to male, female or unspecific causes. Plus, the reduction is still comprised within the historical control data range" (CLH Report for 1-NP, 2023: p. 80).
As it relates to sperm effects, the highest dose tested in Carney et al. (2004), 100 ppm, was a systemically toxic dose (i.e., an appropriate MTD); specifically, current definitions of MTD note avoidance of hypoxia as a consideration in limiting the highest test dose (Lewis et al. 2024: ATTACHED p. 10). If toxicologically significant sperm effects would have occurred, they would have affected fertility, and they would have been present at the highest test dose, even though that parameter was not specifically evaluated in the OECD 422 study. The OECD 422 study did assess the effects of chemical exposure in the premating period in males, and therefore, any effects on sperm quality or quantity would have resulted in fertility changes, and no such changes occurred in the study (Carney et al. 2004: p. 12). Overall, the results of the Carney et al. (2004) study demonstrated that 1-NP does not produce treatment-related reproductive effects. The potential decrease in fertility in the 1-NP study was not statistically significant or outside of historical control data (Carney et al. 2004). The interpretation of the changes in estrous cycle were unclear given the variability in this measure, and there is no evidence for endocrine related activity as a predicate for such an effect. Thus, based on a guideline-compliant OECD 422 study, 1-NP should not be classified as a reproductive toxicant.

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

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	15
Comment received				
FR agrees with the classification of 1-nitropropane as Repr. 2, H361f. FR suggests insisting on the lack of sperm parameters assessments in several studies. FR agrees with the classification of 1-nitropropane 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.				

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
17.04.2024	France	ANGUS Chemie GmbH	Company-Importer	17
Comment received				
Given the Joint submission tonnage band (10-100t), there are no data requirements for repeat-dose toxicity data beyond the available OECD 422 study. Therefore, there is no				

regulatory basis to artificially expand this dataset by using inappropriate read-across to other nitroalkanes with a different metabolism (Nitromethane), as was done in the CLH report. The demonstration that the NM->1-NP read-across disregards RAAF guidelines and toxicological principles can be found in our comments on Carcinogenocity and Reproductive toxicity sections. On this basis, we disagree with the proposed classification as STOT RE 2 and we propose no classification for repeat-dose toxicity based on the available OECD 422 study.

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

Date	Country	Organisation	Type of Organisation	Comment number
18.04.2024	France		MemberState	18
Comment received				
FR agrees with the classification of 1-nitropropane as STOT RE 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 1-nitropropane studies and by read-across analysis with nitroethane and with nitromethane.				

PUBLIC ATTACHMENTS
1. PUBLIC attachments.zip [Please refer to comment No. 3, 8, 11, 14, 17]