

# **Comments on Proposal for Harmonised Classification and Labelling, 1-Nitropropane**

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The conclusions in the Report titled Comments on Proposal for Harmonised Classification and Labelling, 1-Nitropropane are Integral Consulting's professional opinion, as of the time of the Report, and concerning the scope described in the Report. The opinions in the document are based on conditions and information existing at the time the scope of work was conducted and do not take into account any subsequent changes.



Principal

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Date

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## ACRONYMS AND ABBREVIATIONS

1-NP	1-nitropropane
CLH	Harmonized Classification and Labelling
DS	dossier submitter
ECHA	European Chemicals Agency
NE	nitroethane
NM	nitromethane
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
ppm	parts per million
RAAF	Read-Across Assessment Framework
VOC	volatile organic compound
WoE	weight of evidence

## 1 INTRODUCTION

A proposal for Harmonized Classification and Labelling (CLH) of 1-nitropropane (1-NP; EC number: 203-544-9; CAS number: 108-03-2) was recently published and has entered the 60-day comment period. The CLH report notably recommends classifications of Carc. 1B, H350 and Repr. 1B, H360Df for 1-NP. The dossier submitter (DS) used weight of evidence (WoE) and read-across between three nitroalkanes to justify the proposed classifications: 1-NP; nitroethane (NE; EC number: 201-188-9; CAS number: 79-24-3), and nitromethane (NM; EC number: 200-876-6; CAS number: 75-52-5).

The report contained herein presents further justification behind the comments submitted through the European Chemicals Agency (ECHA) portal in response to the Proposal for Harmonised Classification and Labelling for 1-Nitropropane, dated December 2023 (herein referred to as the CLH Report). Further consideration should be given to the carcinogenicity and developmental toxicity classifications of 1-NP.

The opinions summarized herein pertain to Chapters 10.9 (Carcinogenicity) and 10.10 (Reproductive Toxicity) of the CLH report. Specifically, our comments are as follows:

### Chapter 10.9 Carcinogenicity

- 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.

### Chapter 10.10 Reproductive Toxicity

- Read-across from NM used to classify 1-NP does not follow RAAF principles and is scientifically invalid.
- The sperm effects observed in NM 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.

Given the points outlined above and described herein, we propose the classifications outlined in Table 1.

Table 1. DS Proposed and Recommended CLH Classifications for 1-NP

Toxicity Endpoint	DS Proposed Classification	Classification Proposed Herein
Carcinogenicity	Carc. 1B (H350)	No Classification
Reproductive Toxicity	Repr. 1B (H360Df)	No Classification

## 2 CLH REPORT CHAPTER 10.9 – CARCINOGENICITY

The DS has proposed a CLH classification of Carc. 1B (H350) for 1-NP (CLH Report for 1-NP, 2023: pp. 3, 75). 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). DS used read-across from NE and NM data to arrive at this proposed classification. As discussed in greater detail below, this read-across is invalid when considering the differential metabolisms of 1-NP and NM. The available carcinogenicity study for 1-NP and read-across to carcinogenicity data for NE are sufficient to support no carcinogenicity classification for 1-NP.

### 2.1 THE READ-ACROSS FROM NM USED BY DS TO CLASSIFY 1-NP AS CARC. 1B DOES NOT FOLLOW RAAF PRINCIPLES AND IS SCIENTIFICALLY INVALID

The DS has proposed a CLH classification of Carc. 1B (H350) for 1-NP (CLH Report for 1-NP, 2023: pp. 3, 75). In Section 10.9 of the CLH Report, the DS considered available carcinogenicity data for 1-NP and structurally similar compounds NE and NM when applying the Carc. 1B classification. The DS stated of the carcinogenicity data for 1-NP:

“Only one study performed with 1-nitropropane, not following any guideline, is reported in detail and showed a non-significant increased incidence of tumours (benign and malign) in rats (Griffin *et al.*, 1982), but in both exposed and control groups. Two other studies were poorly reported and the only available data mentioned that no increase was seen in the development of tumours in exposed animals, in comparison with the controls. Based on the available information on 1-nitropropane, the carcinogenic potential cannot be assessed properly.” (CLH Report for 1-NP, 2023: p. 74)

The DS proceeded to consider one carcinogenicity study for NE (Anonymous 35, 1986) but 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).

The DS’s assessment of 1-NP carcinogenicity based on read-across to NM carcinogenicity data is inappropriate and invalid for several reasons.

### 2.1.1 The DS Did Not Follow RAAF Guidelines in Its Read-Across Approach

According to the ECHA (2017) RAAF, a category approach to read-across can be used when “*physico-chemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern*” (ECHA 2017: p. 25). According to the RAAF document, substances can be considered as a category if they are structurally similar, with one measure of structural similarity listed as the likelihood of common breakdown products (ECHA 2017: p. 6).

The use of a category approach to read-across to 1-NP is inappropriate based on qualitative differences in the aldehyde metabolites formed and quantitative differences in relative tissue reactivity of those metabolites, between 1-NP and NM. As described in Garnick et al. (2021), cytochrome P450-mediated oxidative denitration 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 formation of formaldehyde as a metabolite by P450-mediated oxidative denitration is a metabolic pathway specific to NM, not 1-NP or other primary nitroalkanes, and is a significant operative mechanism by which NM imparts carcinogenic and genotoxic potential (Garnick et al. 2021, p. 19). See further discussion of the variability of known toxicity of these metabolites in Section 2.1.2.

According to the RAAF, the likelihood of common breakdown products is a measure of structural similarity (ECHA 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. The RAAF also states:

- “Whenever category approach read-across is used, properties of target substances are predicted from properties of source substances within the category. The category hypothesis should apply in an unambiguous manner to all the category members. Only category members that are covered by the category hypothesis can be involved in the read-across” (ECHA 2017: p. 26).
- “The read-across hypothesis should explain how exposure to different compounds causes the same effects or absence of effects. A common mechanism linking the presence of the compounds driving the effects with the prediction needs to be identified, and should also link the structures of the compounds under consideration with the possibility to predict qualitatively similar effects for the target substance” (ECHA 2017: p. 24).
- “Other compounds than those linked to the prediction in the read-across hypothesis may be formed through other (bio)transformation pathways or may be intermediates/metabolites of the identified pathway. Exposure to impurities of the source and/or target substance may also occur. Exposure to these compounds has to be considered in the justification” (ECHA 2017: p. 24).

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 fact, the DS incorrectly relied upon the current categorization of formaldehyde as supporting evidence of 1-NP's potential carcinogenicity. The DS states:

IARC classified nitromethane for carcinogenicity in category 2B 'possibly carcinogenic to humans'. Furthermore, the DS notes as supporting evidence that the metabolism of nitromethane leads to the formation of formaldehyde which has a harmonised classification as Carc. 1B, H350... (CLH Report for 1-NP, 2023: p. 75)

The fact that the DS failed to consider that formaldehyde is not a metabolite or breakdown product of 1-NP shows that the DS has not met the appropriate assessment elements according to the RAAF to justify a read-across. Therefore, the DS's read-across of NM carcinogenicity data when classifying 1-NP as CLH classification Carc. 1B (H350) is invalid and disregards RAAF principles published by ECHA.

### 2.1.2 NM-Specific Metabolite Formaldehyde Makes Read-Across to 1-NP Scientifically Invalid

Cytochrome P450-mediated oxidative denitrification of the primary nitroalkanes, including 1-NP, results in the formation of nitrite and an aldehyde with corresponding chain length. For 1-NP and NM, these aldehyde metabolites are propionaldehyde (CASRN 123-38-6) and formaldehyde (CASRN 50-00-0), respectively. Garnick et al. (2021) note that mutagenicity studies in different *Salmonella* strains demonstrate that the nitrite metabolite is not a major contributor to mutagenicity in nitroalkanes (p. 19). Formaldehyde generation through the oxidative denitrification of NM is a metabolic process that is specific to NM and would not be relevant to the genotoxic or carcinogenic potential of other nitroalkanes such as 1-NP. Formaldehyde has a toxicological profile distinct from that of 1-NP oxidative denitrification metabolite, propionaldehyde. This is evident from a comparison of the current harmonized classification of the two aldehydes, as shown in Table 2.

Table 2. Comparison of Harmonized CLP Classification for Aldehyde Metabolites of 1-NP and NM

Parent Nitroalkane	Corresponding Oxidative Denitrification Metabolite	Harmonized Classification of Metabolite Carcinogenicity / Mutagenicity
1-NP	Propionaldehyde (CAS: 123-38-6)	Not classified / Not classified
NM	Formaldehyde (CAS: 50-00-0)	Carc. 1B / Muta. 2

When applying this logic, NM is an inappropriate read-across to 1-NP because of their different metabolites and their associated toxicity profiles. NM is the only nitroalkane that would

generate formaldehyde as a metabolite. Using NM as a read-across source to any nitroalkane 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 approach.

## **2.2 THE NM STUDIES USED FOR READ-ACROSS DO NOT PROVIDE CONCLUSIVE EVIDENCE OF THE NEED FOR A CARCINOGENICITY CLASSIFICATION FOR NM**

The available NM carcinogenicity studies are not considered relevant for carcinogenicity classification of 1-NP due to invalid read-across. However, even if these NM studies were to be considered in an overconservative approach disregarding both RAAF and toxicological principles, then it should be emphasized that available NM data do not provide conclusive evidence to classify NM as a carcinogen.

### **2.2.1 The NM Rat Carcinogenicity Study Used for Read-Across to 1-NP Does Not Demonstrate a Carcinogenic Potential**

The National Toxicology Program (NTP) study (NTP 1997) of NM carcinogenicity, which the DS relies on for read-across information to 1-NP carcinogenicity, has significant limitations. The NTP (1997) study found that mammary tumors (including fibroadenomas, and fibroadenomas and carcinomas combined) increased in a dose-dependent manner among female F344 rats. However, as noted in Garnick et al. (2021), the incidence of mammary tumors observed in the NTP study 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). Thus, it has been scientifically known for two decades that interpretation of tumor incidence data from this rat strain should be done with great care.

### **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**

The findings in the evaluation of NM carcinogenicity in B6C3F1 mice performed by NTP (1997) are consistent with formaldehyde-related toxicity and further demonstrate that use of NM carcinogenicity data are not appropriate for read-across to 1-NP. We agree with the registrant that harderian tumors have limited relevance for human health (CLH Report for 1-NP, 2023: p. 68, 74; Garnick et al. 2021: p. 6). As the DS notes, liver tumors were only observed in female

mice, but the incidence of these tumors was within the range historically observed in control animals of this strain; therefore, the liver tumors are not treatment-related and do not provide evidence of a carcinogenic potential (CLH Report for 1-NP, 2023: p. 74).

Regarding lung tumors observed in the NTP (1997) mouse study, carcinomas were elevated among the highest dose group of males, outside of the historical control range; however, the same was not observed among female mice (CLH Report for 1-NP, 2023: p. 63–64). Significant increases in combined lung adenoma and carcinoma tumors were only observed above historical control range in the high-dose females (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 in the European Union (U.S. EPA 1991; ECHA 2024).

To the extent tumors were seen outside of the respiratory tract, they were likely secondary to general toxicity. The observation of respiratory tract tumors in the mouse NM cancer study is consistent with the formation of the formaldehyde metabolite and further stress the irrelevance of using these data for read-across to 1-NP.

### **2.3 AVAILABLE DATA FOR 1-NP AND NE ARE SUFFICIENT TO CONCLUDE THAT 1-NP SHOULD NOT BE CLASSIFIED AS A CARCINOGEN**

In a WoE assessment, data for the chemical of interest should be considered first, followed by the most related members of the category (ECHA 2011; WHO 2021). Our prior argument discussed in Section 2.1 indicates that the use of NM data for read-across was inappropriate due to the differential metabolism of 1-NP and NM and the distinct toxicological properties of the NM metabolite formaldehyde. A proper WoE assessment would consider the available data for 1-NP and, at best, consider read-across to the more relevant NE data set.

The DS inappropriately dismissed a relevant inhalation study for 1-NP, Griffin et al. (1982), that found no significant dose-related increase in tumors in male and female rats exposed to 1-NP at concentrations of 0 or 100 ppm and sacrificed at 1, 3, 12, 18, or 21.5 months of exposure (10 rats/sex/group). Griffin et al. (1982) is an informative negative carcinogenicity study. The duration of the study was 21.5 months, which is near the standard length for animal cancer studies of 24 months. Further, doses provided to rats in the study were up to 100 ppm, which is much greater than human-relevant doses and equal to the inhalation dose level Carney et al. (2004) determined to be appropriate for the maximum inhalation dose based on a range-finding study. One limitation of the study was that it had a somewhat limited number of animals per dose group (10 rats/sex/group). However, given that the study evaluated long-term exposure to 1-NP, the compound of interest, as well as an oral carcinogenicity study by Hadidian et al. (1968) that was negative, the Griffin et al. (1982) study presents valuable information that should be considered in a WoE evaluation of 1-NP carcinogenicity.

As a WoE, 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) 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 in the animals and, thus, reached the maximum tolerable dose [MTD] as suggested by OECD 451) and evaluated numbers approaching OECD 451 guidelines at 40/sex/group (OECD 2018; Griffin et al. 1988). As such, the Griffin et al. (1988) 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 exposed to NE.

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).

### **2.3.1 Genotoxicity Findings Do Not Suggest a Genotoxic Potential for 1-NP**

Our conclusion that 1-NP is not classifiable as a carcinogen based on available 1-NP and NE data is further supported by evidence of non-genotoxicity. The CLH report on document 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), 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); the positive response in one assay in lung cells found by Roscher et al. (1990) 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).

### 3 CLH REPORT CHAPTER 10.10 - REPRODUCTIVE TOXICITY

Concerning **development**, the CLH report recommends a classification of Repr. 1B (H360Df) for 1-NP based on evidence of developmental effects in studies of NM (CLH Report for 1-NP, 2023: pp. 101, 103). Regarding this classification, the CLH report stated:

“The classification proposal is based on the read-across with nitromethane as there is no prenatal developmental toxicity study performed on 1-nitropropane and nitroethane. In the available prenatal developmental toxicity study performed with nitromethane (Anonymous 36, 2017), clear evidence of effects on developmental parameters were observed considered not secondary to maternal toxicity which is in line with a classification in category 1B.” (CLH Report for 1-NP, 2023: p. 101)

Concerning **fertility**, the DS also concluded that evidence on the adverse effects on sexual function and fertility (sperm effects) warrants a classification of Repr. 2 (H360f). We believe that the DS did not follow appropriate standards for the assessment of reproductive and developmental toxicity in determining these classifications. The DS did not give proper weight to a guideline-compliant study of 1-NP and inappropriately used read-across to NM data in its assessment.

There is a guideline-compliant study of appropriate design for assessing reproductive and developmental toxicity (OECD 422 study by Carney et al. 2004). Carney et al. (2004) did not show that 1-NP is a reproductive or developmental toxicant. Given the Joint Submission tonnage band (less than 100t/year), this is the only reproduction/development data requirement applicable to 1-NP.

Based on the availability of this adequate study, no classification is warranted for 1-NP. However, because the DS used a questionable read-across approach that was aimed at addressing limitations in the 1-NP study, additional considerations related to the available evidence are described further below.

#### 3.1 READ-ACROSS FROM NM USED TO CLASSIFY 1-NP DOES NOT FOLLOW RAAF PRINCIPLES AND IS SCIENTIFICALLY INVALID

The DS relied upon general systemic toxicity studies that showed effects on sperm for NM and NE as part of the rationale for classification for reproductive effects (Category 2) (CLH Report for 1-NP: pp. 88–89). This read-across approach is not appropriate for several reasons. First, 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, even if that was an operative mode of action, there are quantitative differences in nitrite formation and elimination between NM, NE, and 1-NP. Lai et al. (1982) 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) noted that NM had a unique binding affinity compared to other nitroalkanes in rat liver microsomes.

The read-across approach utilized by the DS in the classification has significant uncertainty and does not fully follow RAAF principles. The RAAF requires demonstrative evidence that two compounds, or a class of compounds, share properties, stating:

- “Whenever category approach read-across is used, properties of target substances are predicted from properties of source substances within the category. The category hypothesis should apply in an unambiguous manner to all the category members. Only category members that are covered by the category hypothesis can be involved in the read-across” (ECHA 2017: p. 26).
- “The read-across hypothesis should explain how exposure to different compounds causes the same effects or absence of effects. A common mechanism linking the presence of the compounds driving the effects with the prediction needs to be identified, and should also link the structures of the compounds under consideration with the possibility to predict qualitatively similar effects for the target substance” (ECHA 2017: p. 24).
- “Other compounds than those linked to the prediction in the read-across hypothesis may be formed through other (bio)transformation pathways or may be intermediates/metabolites of the identified pathway. Exposure to impurities of the source and/or target substance may also occur. Exposure to these compounds has to be considered in the justification” (ECHA 2017: p. 24).

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 metabolite formation. Therefore, the DS’s read-across of NM data when classifying 1-NP as CLH classification Repr. 2 is invalid and disregards RAAF principles published by ECHA.

### **3.2 THE SPERM EFFECTS OBSERVED IN NM STUDIES ARE SECONDARY TO HYPOXIA AND HENCE DO NOT REQUIRE A CLASSIFICATION FOR EFFECTS ON REPRODUCTION**

The available NM and NE studies are not considered relevant for reproductive toxicity classification of 1-NP due to invalid read-across. However, 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 do 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. The mode of action of such effects is not known; therefore, read-across to other compounds such as NM is not appropriate without substantiation of the mode of action and applicability across the proposed class (ECHA 2017). One hypothesis is that the sperm effects may be treatment-related and secondary to hypoxia, an effect of nitrite (Reyes et al. 2012, as described in Garnick et al. 2021: p. 20). Further, the data are not sufficient to demonstrate an effect on the testes, as the repeat dose studies relied upon do not show evidence of the pathology and weight data needed to consider adjustments for body weight and general systemic toxicity. While nitroalkanes as a class might cause sperm changes at some dose, the primary emphasis should be on the data for the chemical being evaluated (i.e., 1-NP). This reflects potential differences in potency (quantitative differences) among related compounds. In particular, there are differences in both nitrite formation kinetics as well as the specific aldehyde formed among the class of nitroalkanes (Sakurai et al. 1980 Lai et al. 1982). Thus, sperm effects related to NM should not be given the same weight as the available 1-NP study. 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: 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, 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), the data for 1-NP specifically support no classification for 1-NP.

### **3.3 AVAILABLE 1-NP DATA ARE SUFFICIENT TO CONCLUDE THAT 1-NP SHOULD NOT BE CLASSIFIED AS A DEVELOPMENTAL TOXICANT**

The DS proposes a classification of Repr. Cat. 1B, H360D for developmental toxicity (CLH Report for 1-NP, 2023: p. 101). The classification proposal is based on a read-across from NM, as 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, the assessment for developmental toxicity presented by the DS does not appropriately weigh the evidence. We recommend no classification based on the absence of observed effects for 1-NP in a guideline compliant OECD 422 study.

A guideline compliant OECD 422 study, Carney et al. (2004) and referenced as “Anonymous 37, 2003” in the CLH report, is available for 1-NP. This study reported a developmental no-observed-adverse-effect concentration of 100 ppm, the highest concentration tested. The DS

suggests that the reliability of this study for classification of 1-NP is limited (CLH Report for 1-NP, 2023: p. 101). However, the presumed basis for this conclusion is not supported by the intended use of the OECD 422 study. The DS concluded that the 1-NP data set lacked an adequate prenatal study and that the highest dose was “a very low dose” (CLH Report for 1-NP, 2023: p. 101). The DS proceeded to inappropriately read-across to data for NM in determining its classification of 1-NP.

An OECD 422 study such as Carney et al. (2004), while not considered as robust as OECD 414, 415, 416, or 443 studies, is designed to screen for potential reproductive effects of a test chemical for the purpose of hazard assessment. OECD states that the 422:

“...comprises a reproduction/developmental toxicity screening test and, therefore, can also be used to provide initial information on possible effects on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition, either at an early stage of assessing the toxicological properties of test chemicals, or on test chemicals of concern. This test does not provide complete information on all aspects of reproduction and development... Moreover, in the absence of data from other reproduction/developmental toxicity tests, positive results are useful for initial hazard assessment and contribute to decisions with respect to the necessity and timing of additional testing.” (OECD 2016, p. 22)

Specifically, the NM study identified methemoglobinemia at 1,200 ppm, and at this concentration, the following developmental effects were observed and concluded to be not related to maternal toxicity: reduced number of litters, fetal malformations, and skeletal malformations. Further, based on other repeated-dose toxicity studies for NM, effects on blood oxygenation (methemoglobinemia) occurred at concentrations as low as 375 ppm (NTP 1997). Thus for NM, the data clearly show that hematological effects, including those related to tissue oxygenation, occur at/or concentrations well below developmental effects. The embryo and fetal toxicity endpoints observed for NM, or corollary endpoints, are evaluated as part of the OECD 422 study design, including evaluation of litter size and pup abnormalities (OECD 2016). Therefore, negative findings observed in Carney et al. (2004) should support hazard assessment findings.

With regard to the DS’s primary criticisms of the Carney et al. (2004) 1-NP study, both the Carney et al. (2004) study and the prenatal development study of NM relied upon by the DS cover an overlapping exposure period. In fact, the 1-NP study covers pre-mating and post-parturition periods not covered in the NM study. Thus, in terms of periods of susceptibility, the 1-NP study is superior. Further, while Carney et al. (2004) did not assess fetal variations, there are many effects that were fully examined in both the NM and 1-NP studies. 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. While this study did not observe “severe” or frank observable clinical effects at 100 ppm, the study was sufficient. This reflects two key points. 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), 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 1-NP at high doses (Garnick et al. 2021). Although, the 100 ppm concentration in the Carney et al. study did not show evidence of methemoglobinemia or hematological changes, the goal of the OECD 422 study is to test as close to but before the onset of such effects. Therefore, the highest concentration tested is appropriate in the consideration of avoiding hypoxia. Further, the 100 ppm concentration did induce target organ effects, including decreased bodyweight in males and nasal histopathological changes in females suggestive of doses that would induce animal stress.

Overall, Carney et al. (2004) concluded that there was no evidence of selective developmental toxicity in 1-NP. A slight decrease in litter size was observed, but the study authors attributed these developmental effects to maternal toxicity and/or stress (p. 12).

### **3.4 AVAILABLE 1-NP DATA ARE SUFFICIENT TO CONCLUDE THAT 1-NP SHOULD NOT BE CLASSIFIED AS A REPRODUCTIVE TOXICANT**

The assessment for reproductive toxicity and fertility was recommended as Category 2 (H361f for adverse effects on sexual function and fertility) by the DS (CLH Report for 1-NP, 2023: p. 93). The DS considered the previously discussed guideline-compliant OECD 422 study by Carney et al. (2004), cited as “Anonymous 37. 2003” in the CLH report, as well as data for NE and NM in its assessment. The proposed classification is based on observations of sperm-related effects, potential effects on testes, indications of decreased fertility (but within historical control data), and impacts to the estrous cycle. However, the DS relied upon data from the entire class of nitroalkanes rather than relying primarily on the guideline-compliant OECD 422 study.

An OECD 422 study such as Carney et al. (2004), while not considered as robust as OECD 414, 415, 416, or 443 studies, is designed to screen for potential reproductive effects of a test chemical for the purpose of hazard assessment. OECD states the 422:

“comprises a reproduction/developmental toxicity screening test and, therefore, can also be used to provide initial information on possible effects on male and

female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition, either at an early stage of assessing the toxicological properties of test chemicals, or on test chemicals of concern. This test does not provide complete information on all aspects of reproduction and development... Moreover, in the absence of data from other reproduction/developmental toxicity tests, positive results are useful for initial hazard assessment and contribute to decisions with respect to the necessity and timing of additional testing” (OECD 2016, p. 22).

Therefore, negative findings observed in Carney et al (2004) should support hazard assessment findings.

Carney et al. (2004) 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 of 1-NP in Sprague-Dawley rats exposed at concentrations of 0, 25, 50, or 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: 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.

## 4 CONCLUSION

In conclusion, we encourage the DS to reconsider the proposed classifications for 1-NP regarding carcinogenicity and reproductive toxicity. Specifically, we believe that the read-across approach used by the DS is not in line with scientific and RAAF principles and that a scientifically sound WoE approach warrants “no classification” for carcinogenicity and reproductive toxicity of 1-NP.

Overall, the read-across approach between the three primary nitroalkanes utilized by the DS did not respect RAAF principles and should not be considered valid in the classification of 1-NP as Carc. 1B and Repr. 1B. There are qualitative and quantitative differences between the three primary nitroalkanes; hence, direct use of NM data is not appropriate to conclude for 1-NP. Data for 1-NP itself should take precedence over data for analogs NM and NE, because they derive from studies of adequate design and quality to assess carcinogenicity and reproductive hazards. The DS did not seem to take into account a detailed WoE analysis presented in a critical review published in 2021 in the peer-reviewed *Journal of Applied Toxicology* (Garnick et al. 2021).

Considering mode of action is critical for each endpoint. In the case of 1-NP, data should be weighed as 1-NP > NE > NM. There is scientific evidence that the differential metabolisms of the three nitroalkanes contribute to distinct toxicological profiles for each compound. Additionally, there may be quantitative differences in rate and degree of metabolism of the compounds that have not been assessed (yielding different systemic dose patterns of nitrite, a key metabolite in the mode of action for potential reproductive effects). In addition, as noted by the DS, the aldehyde metabolite arising from each compound differs, indicating a qualitative difference in potential effect potency for carcinogenicity.

## 5 REFERENCES

Carney, E.W., C.L. Zabloutny, A.K. Andrus, S.M. Krieger and K.E. Stebbins. 2004. 1-Nitropropane: A Combined Repeated Inhalation Exposure Study with the Reproduction/Developmental Toxicity Screening Test in CD Rats. R&D Report, the Dow Chemical Company. **Cited as “Anonymous 37, 2003” in CLH Report.**

CLH Report for 1-NP. 2023. CLH Report: Proposal for Harmonised Classification and Labelling; Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2; International Chemical Identification: 1-Nitropropane. Submitted by Belgian Federal Public Service Health, Food Chain Safety and Environment, Risk Management Service. Version 4; December 2023.

ECHA. 2011. Guidance on information requirements and chemical safety assessment Part B: Hazard assessment. Reference ECHA-11-G-16-EN. European Chemicals Agency. December.

ECHA. 2017. Read-Across Assessment Framework (RAAF). Reference ECHA-17-R-01-EN. European Chemicals Agency. March.

ECHA. 2024. Summary of Classification and Labelling for formaldehyde (EC number: 200-001-8; CAS number: 50-00-0). Available: <https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/55163>

Garnick, L., C. Gillie, J. Kozal, A. Monnot, P. Spencer, J. Quinn, and A. Maier. 2021. Hazard characterization of carcinogenicity, mutagenicity, and reproductive toxicity for short chain primary nitroalkanes. *J. Appl. Toxicol.* 2021;1–27. DOI: 10.1002/jat.4169.

Griffin T.B., A.A. Stein, and F. Coulston. 1982. Inhalation exposure of rats to vapors of 1-nitropropane at 100 ppm. *Ecotox. Environ. Safety* 6: 268-282.

Griffin, T.B., A.A. Stein, and F. Coulston. 1988. Chronic inhalation exposure of rats to vapors of nitroethane. *Ecotox. Environ. Safety* 16(1): 11–24. **Cited as “Anonymous 35, 1986” in CLH Report.**

Hadidian, Z., N. Fredrickson, E.K. Weisburger, J.H. Weisburger, R.M. Glass, and N. Mantel. 1968. Tests for chemical carcinogens. Report on the activity of derivatives of aromatic amines, nitrosamines, quinolines, nitroalkanes, amides, epoxides, aziridines, and purine antimetabolites. *J. Natl. Cancer Inst.* 41: 985-1036.

Lai, D. Y., Y.T. Woo, J.C. Arcos, and M.F. Argus. 1982. Nitroalkanes and nitroalkenes: Carcinogenicity and structure activity relationships: Other biological properties: Metabolism: Environmental significance. U.S. Environmental Protection Agency, Chemical Hazard identification Branch.

Lewis, R.W., A.K. Andrus, J. Arroyo, S. Brescia, P.A. Botham, M. Corvaro, G.P. Daston, T. Hofmann, C. Rodriguez, F. Sewell, B. van Ravenzwaay, K. Wiench, and S. Marty. 2024. Considerations for the development of guidance on dose level selection for developmental and reproductive toxicity studies. *Regulatory Toxicology and Pharmacology* Volume 148: 105585, [bhttps://doi.org/10.1016/j.yrtph.2024.105585](https://doi.org/10.1016/j.yrtph.2024.105585).

NTP. 1997. NTP toxicology and carcinogenesis studies of nitromethane (CAS No. 75-52-5) in F344/N rats and B6C3F1 mice (inhalation studies). Technical report series, 461, 1–289. National Toxicology Program.

OECD. 2016. *Test No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test*, OECD Guidelines for the Testing of Chemicals, Section 4. Organisation for Economic Co-operation and Development Publishing, Paris.

OECD. 2018. *Test No. 451: Carcinogenicity Studies*, OECD Guidelines for the Testing of Chemicals, Section 4. Organisation for Economic Co-operation and Development Publishing, Paris.

Reyes, J.G., J. G. Farias, S. Henriquez-Olavarrieta, E. Madrid, M. Parraga, A.B. Zepeda, and R.D. Moreno. 2012. The hypoxic testicle: Physiology and pathophysiology. *Oxidative Med. Cell. Longevity*. 2012: 1-15.

Roscher, E., K. Ziegler-Skylakakis, and U. Andrae. 1990. Involvement of different pathways in the genotoxicity of nitropropanes in cultured mammalian cells. *Mutagenesis*. 5: 375-380.

Sakurai, H., G. Hermann, H.H. Ruf, and V. Ullrich. 1980. The interaction of aliphatic nitro compounds with the liver microsomal monooxygenase system. *Biochemical Pharmacology* 29(3): 341–345.

WHO. 2021. Framework for the use of systematic review in chemical risk assessment. World Health Organization, Geneva.

U.S. Environmental Protection Agency (U.S. EPA). 1991. Formaldehyde; CASRN 50-00-0. Integrated Risk Information System (IRIS) Chemical Assessment Summary. U.S. EPA National Center for Environmental Assessment.