

# **Comments on Proposal for Harmonised Classification and Labelling, Nitromethane**

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The conclusions in the Report titled Comments on Proposal for Harmonised Classification and Labelling, Nitromethane 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
CLP	Classification, Labelling and Packaging
DS	dossier submitter
MTD	maximum tolerable dose
NE	nitroethane
NM	nitromethane
NTP	National Toxicology Program
ppm	parts per million
WoE	weight of evidence

## 1 INTRODUCTION

A recently published proposal for Harmonized Classification and Labelling (CLH) of Nitromethane (NM; EC number: 200-876-6; CAS number: 75-52-5) notably recommended classification of NM as Carc. 1B, H350 and Repr. 1B, H360Df. The dossier submitter (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.

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 mammary tumors observed in the National Toxicology Program (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.

### Chapter 10.10 Reproductive Toxicity

- The studies used to classify NM as a developmental toxicant exceed the maximum tolerated dose (MTD) and are not reliable for classification.
- The sperm effects observed in NM studies are secondary to systemic toxicity in studies without investigation of reproductive function and hence do not require a classification for effects on reproduction.

Given the points summarized above and discussed in greater detail in this report, the available NM data do not support the classification of NM as Carc. 1B, H350 and Repr. 1B, H360Df.

## 2 CLH REPORT CHAPTER 10.9 – CARCINOGENICITY

The DS has proposed a CLH classification of Carc. 1B (H350) for NM (CLH Report for NM, 2023: pp. 3, 76). The DS stated the following regarding the selection of Carc. 1B classification:

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

In a second independent study in rats (Anonymous 34, 1990), no increase in treatment-related tumours was induced but a reason could be that the doses used in this study were not high enough. The susceptibility of the two different strains to chemical carcinogenesis in the mammary gland was quoted similar (Wood *et al.*, 2002).” (CLH Report for NM, 2023: p. 75)

As per Classification, Labelling and Packaging (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 NM, 2023: p. 74). Although the DS proposed to classify NM, nitro ethane (NE), and 1-nitropropane (1-NP) as a class, the classification of NM was based solely on data for NM, as the DS deemed the available negative carcinogenicity assays of NE and 1-NP as “uninformative” (CLH Report for NM, 2023: p. 75). It is worth noting that the negative carcinogenicity assays for NE and 1-NP only add to the WoE that NM is not likely to be carcinogenic (ECHA 2011; WHO 2021).

The available NM carcinogenicity studies have limitations that may preclude their consideration in a WoE approach classification of NM carcinogenicity. This WoE approach to NM classification is described in greater detail in Garnick *et al.* (2021), which concluded:

“1B is not appropriate for NM with respect to carcinogenicity. Of the three primary nitroalkanes, only NM was shown to induce tumors in vivo following chronic exposure, which was hypothesized to be due to impurities in the NM used in each study, or differential strain sensitivities to mammary tumor formation. Furthermore, rat mammary and mouse harderian tumors are likely not relevant to humans, and the reported lung and liver tumors in mice were equivocal in their dose response and statistical significance” (p. 24).

Overall, the available NM data do not provide conclusive evidence to classify NM as Carc. 1B based on the requirements stated above.

## **2.1 THE MAMMARY TUMORS OBSERVED IN THE NTP (1997) NM RAT CARCINOGENICITY STUDY DO NOT DEMONSTRATE A CARCINOGENIC POTENTIAL**

The NTP (1997) study of NM carcinogenicity has significant limitations. The study found that mammary tumors increased in a dose-dependent manner among female F344 rats. However, the incidence of these mammary tumors stayed within the historical control range (Garnick et al. 2021: pp. 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.

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 parts per million [ppm] in Griffin et al. 1996; 180 ppm in NTP 1997) and at comparable dosing regimens.

## **2.2 THE NTP (1997) NM MOUSE CARCINOGENICITY STUDY SHOWS EFFECTS SECONDARY TO LOCAL TOXICITY THAT WOULD NOT BE 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 and further demonstrate that use of NM carcinogenicity data is not appropriate for read-across to NE. We agree with the DS that harderian tumors have limited relevance for human health (CLH Report for NM, 2023: pp. 69, 76; Garnick et al. 2021: p. 6). As the DS notes, the incidence of the observed liver tumors was within the historical control range for this strain, so these liver tumors were not treatment-related and do not provide evidence of a carcinogenic potential (CLH Report for NM, 2023: p. 75).

Increased lung tumors were observed in the NTP (1997) mouse study at the high dose of 750 ppm (carcinomas in males, combined lung adenoma and carcinoma in females; CLH Report for NM, 2023: p. 63). However, the 750-ppm high dose was associated with respiratory tract non-neoplastic effects, suggesting that pulmonary tumors were 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). Additionally, there was no clear concentration-response pattern for lung tumor incidence, and the incidence of lung tumors in male and female mice was comparable to the historical control range (Garnick et al. 2021: p. 6). To the extent tumors were seen outside of the respiratory tract, they were likely secondary to general toxicity.

The lung tumors observed at high doses in the NTP (1997) mouse study reflect a general response to significant cytotoxic insult. Thus, the available NM studies are not sufficiently informative for cancer classification.

### **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. (2021) concluded the compound was not genotoxic.

### 3 CLH REPORT CHAPTER 10.10 – REPRODUCTIVE TOXICITY

Concerning **development**, the CLH report recommends a classification of Repr. 1B (H360D) for NM (CLH Report for NM, 2023: pp. 4, 103), stating:

“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 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), stating:

“The classification proposal for sexual function and fertility of nitromethane, nitropropane and nitroethane is based on the overall WoE from all category members. There is no EOGRTS or 2-generation study on any of the category member, and thus only limited aspects of potential effects on sexual function and fertility have been investigated in the available data set. However, spermatotoxic effects were reported on nitromethane (90-day NTP studies in rats and mice) and nitroethane (90-day NTP study in rats) and these findings are supported by nitrate/nitrite-mediated spermatotoxic and fertility related effects involving NO redox cycle. As indicated above, nitrite is the common metabolite for nitromethane, nitroethane, and 1-nitropropane. In addition, the OECD TG 422 on 1-nitropropane showed that 2 females at the mid- and high dose groups failed to become pregnant. Overall, these data are considered to support Repr. 2; H361f for nitromethane, nitroethane and 1- nitropropane and these classifications are proposed in individual dossiers.” (CLH Report of NM, 2023: p. 12)

These classifications are not appropriate based on the nature of the NM studies, which exceeded the MTD values and did not adequately consider testing limits that address concern for hypoxia. Thus, the available NM studies are not sufficiently informative for classification.

#### 3.1 THE STUDIES USED TO CLASSIFY NM AS A DEVELOPMENTAL TOXICANT EXCEEDED THE MTD AND ARE NOT RELIABLE FOR CLASSIFICATION

The DS relied primarily on findings of developmental effects in a prenatal developmental toxicity study (cited as Anonymous 36, 2017 in the CLH report) in its classification of NM as a Repr. 1B developmental toxicant, stating, “All these developmental effects appeared at the highest dose only (1200 ppm, equivalent to 2.99 mg/L) in the absence of dose-relationship or severe maternal toxicity” (CLH Report of NM, 2023: p. 102). Methemoglobinemia was also

identified 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. 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 that concentration or at concentrations well below those producing developmental effects. 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 NM at high doses (Garnick et al. 2021).

### **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**

The DS focused on sperm effects as the primary basis for the reproductive toxicity classification. One hypothesis is that the sperm effects were secondary to hypoxia, an effect of nitrite (Reyes et al. 2012, as described in Garnick et al. 2021: p. 20). In addition, the observed sperm effects for NM and NE 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 the criteria to classify NM for fertility effects, in absence of evaluation of reproductive function.

## 4 CONCLUSION

In conclusion, we encourage the DS to reconsider the proposed classifications for NM regarding carcinogenicity and reproductive toxicity.

For Carc. 1B classification, the current CLP criteria require that:

“... a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites” (CLH Report for NM, 2023: p. 74).

The DS proposed classification of NM as Carc. 1B “[b]ased on the fact that nitromethane induced an increased incidence of mammary tumours in female rats (statistically significant in carcinoma at the highest dose and in combination of benign and malignant tumours at the two highest doses which was also dose-dependent) (NTP, 1997)” (CLH Report for NM, 2023: p. 75). However, the findings of the NTP (1997) rat study are of limited relevance due to the high background rate of tumors within the F344 strain used in the study. Furthermore, such tumors were not observed in a comparable study in Long-Evans rats (Griffin et al. 1996). Overall, the limitations of the NTP (1997) rat and mouse studies preclude their results from being considered in the WoE classification of NM, leading to insufficient data to classify NM as Carc. 1B based on the current CLP criteria.

Furthermore, the available NM studies are not sufficiently informative for classification of NM as Repr. 1B. The NM studies considered by the DS in its proposed classification exceeded the MTD values and did not adequately consider testing limits that address concern for hypoxia.

## 5 REFERENCES

- CLH Report for NM. 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: Nitromethane. 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. 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., F. Coulston, and A.A. Stein. 1996. Chronic inhalation exposure of rats to nitromethane. *Ecotoxicology and Environmental Safety* 34(2): 109–117. **Cited as “Anonymous 34, 1990” in the CLH Report.**
- 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.
- 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.
- U.S. EPA. 1991. Formaldehyde; CASRN 50-00-0. Integrated Risk Information System (IRIS) Chemical Assessment Summary. U.S. Environmental Protection Agency National Center for Environmental Assessment.
- WHO. 2021. Framework for the use of systematic review in chemical risk assessment. World Health Organization, Geneva.