Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

dinitrogen oxide

EC Number: 233-032-0
CAS Number: 10024-97-2

CLH-O-0000007281-79-01/F

Adopted

16 March 2023

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DINITROGEN OXIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: Dinitrogen oxide
EC number: 233-032-0
CAS number: 10024-97-2
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
11.07.2022	Germany		MemberState	1
Comment received				
It is important to note that the exposure levels tested in rats and demonstrating reproductive toxicity (up to 750 000 ppm) are in the range of concentrations used for patients which were undergoing surgeries under N2O anaesthesia (60 % N2O).				
Dossier Submitter's Response				
Thank you for your comment and for pointing out that the exposure levels used in animal testing are not unrealistically high levels as they are in the range of concentration used in human. As an example, in France, MEOPA is used as a mixure of 50% N_2O (500,000 ppm) and 50% O_2 for anaesthesia.				
RAC's response				
Thank you for the comment. The arguments have been taken into account in the RAC opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	United Kingdom		Individual	2
Comment received				
N2O has been used professionally for more than a century and its use as intended is not injurious. Misuse or overuse of N2O as a drug (recreational or otherwise) can be injurious however the changing of chemical classification is an inappropriate (and almost certainly ineffective) method of reducing that misuse or overuse. More appropriate methods exist for the controlled use of substances.				
Dossier Submitter's Response				
Thank you for your comment. Thank you for highlighting the growing concern about the abuse and misuse of nitrous oxide. We agree that CLP is only applicable to intended use or reasonably foreseenable conditions of misuse of a substance.				

RAC's response
RAC notes that classification is not intended to limit or prevent misuse. However it could support secondary risk management measures. RAC classifies on basis of the information that is provided to the committee.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	Austria	KAYSER Berndorf GmbH	Company-Manufacturer	3

Comment received

From the available animal data, all of the studies have limitations, were not conducted to GLP or any relevant test guideline. Many of the studies were undertaken with a single dinitrogen oxide dose group, thereby unable to establish a concentration-response relationship. Animal group numbers were generally below the recommended test guideline group numbers, thereby making statistical analysis (if undertaken) difficult to interpret. Where adversity was observed this occurred at concentrations that exceeded the maximum recommended concentration for repeat dose inhalation studies (20,000 ppm), without consideration of hypoxic effects, other plausible modes of action (as detailed in Boobis et al (2008). IPCS framework for analyzing the relevance of noncancer mode of action for humans. Crit. Rev. Toxicol. 38, pp 87-96) or maternal toxicity. Collectively concerns are raised over the reliability of the data used to derive the classification.
The human data considered as a weight of evidence approach for fertility effects are of limited scientific use and do not consider co-exposure to other factors which may impair fertility.
From the animal and human data presented uncertainties exist, with the data deemed insufficient to serve as a basis for classification for sexual function, fertility or developmental toxicity (Repr. 1B, H360fD).
For more specific information please see the attached position paper.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment ERM_KAYSER_Position_Paper.pdf

Dossier Submitter's Response

Thank you for your comment and the in-depth analysis of the studies available in the CLH report.

Fertility and sexual function

1. Data quality

We acknowledge that there are limitations in the available database investigating the effect of nitrous oxide on sexual function and fertility (e.g. low number of animals, lack of statistical analysis, lack of details in study methodology).

Although the database is limited, positive results for sexual function and fertility were obtained in 5 out of 5 studies at relevant dose levels. We acknowledge that in one of the study, statistical significance was not demonstrated but higher dose levels may have been tested (Holson et al., 1995).
In addition, as a result of the low number of animals used per group in some of the published studies, a lower sensitivity could have been anticipated. In contrast, clear positive

results, toxicologically relevant, were obtained (e.g. 50% decreased in fertility in exposed group compare to 100 % in controls) in a coherent manner among the available studies. Therefore, based on weight-of-evidence, effect on sexual function and fertility have been observed with dinitrogen oxide.

2. Maternal toxicity

The following issue was raised in your comment: lack of maternal toxicity reporting to exclude that the observed effects are secondary non-specific consequence of other toxic effects.

We acknowledge that the available data on sexual function and fertility provide low details on general toxicity. Dose levels indicating effects on fertility and sexual function in rats were up to 300,000 ppm. In the developmental toxicity studies (see below), no maternal toxicity was reported ≤ 350,000 ppm in rats (Mazze et al., 1982, 1984 and 1987). Marked toxicity, as described in the guidance, can therefore be reasonably excluded and the effect on oestrous cycle and fertility is not expected to be a consequence of other unspecific toxic effect. Although it could be speculated that hypoxia may be a mode of action for fertility effects, there is no information to support this hypothesis (e.g. PaO_2 and PCO_2 were not measured in the studies). In addition, in Vieira et al., 1983a, females were not exposed to dinitrogen oxide (male only did), so, hypoxia cannot explain the observed effects on litter size in this study and may also not be responsible of the other effects in the other reported studies. As suggested in our report, the effect of dinitrogen oxide on ovary and implantation rate may also be related to the disturbance of folate metabolism induced by dinitrogen oxide. However, in the available database, there are no mechanistic data to support this MoA for fertility and sexual function.

Overall, the decreased fertility and oestrous cycle changes observed consistently in females rats in two studies lead to clear concern on female fertility. In addition, the effects observed on testis and spermatogenesis in Kripke et al., 1976 and the decrease in litter size in Vieira et al., 1983a support potential male fertility effects. Category 2 is considered more appropriate than category 1B due to the limitation in the database and the limited number of parameters investigated in the studies. Dinitrogen oxide warrant to be **classify as Repr. 2, H361f.**

Developmental toxicity

1. Quality of the experimental database

i) Concentration used in the developmental toxicity studies

It is stated in your comment that the effective concentration inducing resorptions and malformations (≥500,000 ppm) is 25-fold greater than the maximum recommended concentration for inhalation studies as recommended in OECD TG).

We would like to point out that in the OECD TG 414 on prenatal developmental toxicity study, the following recommendations are made for the top dose level: *"Expected human*

exposure may indicate the need for a higher oral dose level to be used in the limit test. For other types of administration, such as inhalation or dermal application, the physical chemical properties of the test chemical often may indicate the maximum attainable level of exposure".
In addition in section 3.7.2.5.7 of the CLP regulation, for the classification for reproductive toxicity, it is stated that "*There is general agreement about the concept of a limit dose, above which the production of an adverse effect is considered to be outside the criteria which lead to classification, but not regarding the inclusion within the criteria of a specific dose as a limit dose. However, some guidelines for test methods, specify a limit dose, others qualify the limit dose with a statement that higher doses may be necessary if anticipated human exposure is sufficiently high that an adequate margin of exposure is not achieved. Also, due to species differences in toxicokinetics, establishing a specific limit dose may not be adequate for situations where humans are more sensitive than the animal* model."
In these recommendations, no limit dose by inhalation are recommended. In addition, in the case of dinitrogen oxide exposure levels higher than 20,000 ppm could be anticipated in human. Therefore, the effects observed at high dose levels with dinitrogen oxide are not considered outside the criteria which lead to classification.

At lower dose levels, some evidence of embryofetal toxicity was also observed. In Vieira et al., 1980, a decrease in litter size, an increase in resorptions and skeletal malformations were induced by dinitrogen oxide at 1000 ppm following exposure during the whole gestation period. We disagree that the effects observed in Vieira *et al.,* 1980 are contradictory with Vieira et al., 1983 or Holson et al., 1995 as different dosing regiments were used in that study (6h per day *vs* 23h per day).

ii) OECD TG

The available studies were not performed according to OECD TG. However, we would like to highlight the high number of studies reporting constitently the developmental effects of dinitrogen oxide at consistent dose levels. Although some studies used single dose levels, dose-response was investigated in some of them (Mazze et al., studies). In addition, high number of animals were used. The database is therefore considered robust in a WOE approach.

2. Maternal toxicity

The following issue was raised in your comment: it can be reasonably expected that dams exposed to 50%-75% (500,000 -750,000 ppm) nitrous oxide are sedated and faced hypoxia, explaining the reduced body weight in dams, reduced pup weight at birth and the increased resorptions.

Maternal toxicity was not reported in all the available published paper. However, when available, at the highest dose tested (750,000 ppm), the following effects were reported: drowsiness, impaired motor coordination, body weight, food and water consumption decrease.
There are evidence in the dossier that the developmental effects (malformations, resorptions) are not secondary non-specific consequences of other toxic effects induced

by dinitrogen oxide (maternal toxicity, hypoxia). Exposure window leading to the developmental effects of dinitrogen oxide has been investigating. In Fujinaga et al., 1989, dams were exposed 24h at GD 6, 7, 8, 9, 10 11 or 12 of pregnancy (plug day = day 0 of pregnancy) at the same concentration level of 600,000 ppm. An increase in resorption and major malformations were only noted in dams exposed at GD8, 9 or 11. As the effects were specific to some exposure window only, this may suggest that the increase in resorptions was not related to the maternal toxicity observed at this dose level but rather to a specific effect occurring during this period of development. It may also been noted that at 500,000 ppm, where only decreased body weight gain was reported, teratogenicity of dinitrogen oxide was already observed and may not be explained by body weight changes. In addition, in Mazze et al., 1984, immediately following exposure to 250,000 ppm dinitrogen oxide, withholding food and water during exposure to 250,000 ppm dinitrogen oxide did not result in an increased incidence of abnormalities, suggesting that food deprivation was not related to the increase in early resorptions, late resorptions or malformation observed in the study with dinitrogen oxide at 750,000 ppm.

In addition, in Lane et al., 1980, rats were exposed to xenon or 750,000 ppm dinitrogen oxide for 24 hours on GD 9. Foetal resorption, delayed maturation and anomalies to the skeletal systems were only observed with dinitrogen oxide and not xenon, suggesting that the observed effects are related to the substance itself rather than an anaesthetic mechanism.

With regards to hypoxia, nitrous oxide was usually reported to be mixed with oxygen in the developmental studies. O_2 concentration was monitored continuously and CO_2 concentration were measured and checked during exposure (e.g. Mazze et al.). No respiratory ditress or death from asphyxia was reported in the reliable published studies. Involvement of hypoxia has only been discussed in Shah et al., 1979 were dose levels up to 950,000 ppm were used and were clearly hypoxic. It may also be noted that the teratogenic findings reported in your comment to be induced by hypoxia (degeneration in the basal ganglia, cerebral cortex, anterior horn of the spinal cord, cleft lips) are not in line with the malformations observed with dinitrogen oxide.

As corrected maternal body weight was not available in any studies we acknowledge that it is not possible to decide if the decrease of body weight reported in dams was due to dinitrogen oxide toxicity only or increased resorptions only or both.

Overall, based on the clear embryo-lethality observed following dinitrogen oxide exposure, **not secondary to unspecific maternal toxicity**, dinitrogen oxide warrants to be classify as Repr. 1B, H360D for developmental toxicity.

3. Human data

In human, some studies indicated that dinitrogen oxide may induce congenital abnormalities or reduction of birth weight following high exposure (no measurements available) to dinitrogen oxide or in the absence of appropriate scavenging systems. Nevertheless, we agree that the interpretation of the human data is difficult due to potential co-exposure, the absence of reliable characterisation of exposure, and the absence of

adjustment for potential other risk factors. As discussed in the CLH report, we agree that dinitrogen oxide does not fulfill the criteria for category 1A.
RAC's response
Thank you for the comment. We support the response from the DS and these arguments have been taken into account in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	Hungary	LISS Zrt.	Company-Manufacturer	4
Comment received				
From the available animal data, all of the studies have limitations, were not conducted to GLP or any relevant test guideline. Many of the studies were undertaken with a single dinitrogen oxide dose group, thereby unable to establish a concentration-response relationship. Animal group numbers were generally below the recommended test guideline group numbers, thereby making statistical analysis (if undertaken) difficult to interpret. Where adversity was observed this occurred at concentrations that exceeded the maximum recommended concentration for repeat dose inhalation studies (20,000 ppm), without consideration of hypoxic effects, other plausible modes of action (as detailed in Boobis et al (2008). IPCS framework for analyzing the relevance of noncancer mode of action for humans. Crit. Rev. Toxicol. 38, pp 87-96) or maternal toxicity. Collectively concerns are raised over the reliability of the data used to derive the classification. The human data considered as a weight of evidence approach for fertility effects are of limited scientific use and do not consider co-exposure to other factors which may impair fertility. From the animal and human data presented uncertainties exist, with the data deemed insufficient to serve as a basis for classification for sexual function, fertility or developmental toxicity (Repr. 1B, H360fD). ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Comments on Proposal for Harmonised Classification and Labeling of Dinitrogen Oxide 7July2022.pdf				
Dossier Submitter's Response				
Thank you for your comment, see response to comment number 3.				
RAC's response				
Thank you for the comment. The arguments have been taken into account in the RAC opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	United Kingdom		Individual	5
Comment received				
Commenting upon the CLH justification 4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL Action is required at Community level based on the need to classify for CMR endpoints. Furthermore, self-classification STOT SE 3 for brain by inhalation is not sufficient and justify the need for action at Community level since, based on animal and human data, there are evidence that the substance shall be classified for STOT RE 1 for the nervous system in addition to classification STOT SE 3 for narcosis.				

My comment is
The wording in the justification is misleading as it implies there is evidence of reproductive harm in humans which is untrue.
The evidence of reproductive harm quoted in CLH appendices relates to massive overdosing (well beyond any professional dosing that might be proscribe to a human) of animals in scientific trials.

The only evidence quoted in CLH is of harm in humans associated to withdrawal from habitual use of N2O and is mainly concerning the numbness of feeling in limbs. The misuse/overuse of N2O as a recreational drug

As the CLH report goes on the state, "N2O is used for more than 150 years in surgery as an adjuvant in inhalational general anaesthesia. The substance is also used for pain relief during delivery or for short analgesia during minor medical procedure (e.g. dentistry, emergency, veterinary medicine). The substance is commonly used in combination with other anaesthetics.". A drug leading to reproductive harm would have been noted / researched during that 150 years.

Dossier Submitter's Response

Thank you for your comment. The large amount of scientific published paper on dinitrogen oxide in animals and/or human provide evidence that the substance warrant to be classify as a reproductive toxicant, and as STOT RE and STOT SE.
It may be noted that the proposed classification on reproductive toxicity in category 1B is largely based on animal data.

RAC's response

Thank you for the comment.

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	Belgium	EIGA	Industry or trade association	6

Comment received

The proposed harmonised classification on health effects is different from the classification used by our members. As required by REACH, dinitrogen oxide was registered by our members. A Chemical Safety Report and a classification were made at that time following a robust assessment of the available studies. The EIGA members have monitored the evolution of data published with respect to health and environmental inpact with a view to reconsider the classification. It is our opinion that no research has been published that would change the classification of N2O.

ERM, a consultancy firm sponsored by Kayser, reviewed in detail the literature study and annex mentioned on the webpage 'Registry of CLH intentions until outcome – dinitrogen oxide'. Report No : 0649151-Tox 1 from 7 july 2022 : "Proposal for Harmonised Classification and Labelling of Dinitrogen oxide : Position paper discussing reproductive and developmental CLH proposals." We have read this review via one of our members. EIGA will not copy the exact report with its comments but wishes to emphasize that our members support the comments contained within the report from ERM.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment N2O harmonized classification_EIGA comments.pdf

Dossier Submitter's Response
Thank you for your comment. Please see response to comment number 3. Registration dossier has been taken into account while preparing the CLH proposal.
RAC's response
Thank you for the comment. The arguments have been taken into account in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
13.07.2022	Austria	iSi GmbH	Company-Downstream user	7

Comment received

The data evaluated by ANSES was summarized on pp. 10 – 44 of the Classification proposal. As detailed in the objection attached as pdf file, the quality of the database is considered not sufficient for classification according to regulation (EC) No. 1272/2008.
Arguments for Non-Classification of N2O:
• Data quality is not sufficient
• No consistent results
• Methodological shortcomings: e.g.:
o low number of animals,
o no valid description of Inhalation procedure,
o exposure to only one concentration of N2O
o wrong exposure duration (to detect effects)
o very limited number of parameters observed
o missing statistical analysis
o relevance of effects not shown
• No maternal toxicity reported (no toxic signs except death were reported)
o secondary effects are more likely than a specific effect on reproduction or development
• No prove of mode of action due to effect that can be related to hypoxia
o proposed mode of action (cobalamin deficiency) was not demonstrated.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Position paper_Harmonised Classification and Labeling_iSi GmbH.zip

Dossier Submitter's Response

Thank you for your comment. Please note that the consideration of the economic impact of a proposed classification for a substance is not within the scope of CLH.

Amendment in table 10 of the CLH report.
Thank you for the amendments provided in the table 10 of the CLH report.
Please note the followings:
- Exposure gestation days have been harmonised considering plug day = GD0 in the table available in the CLH report. Gestation days as published in the studies are available in the Annex I of the CLH report (Plug days were either GD0 or GD1).
- In Fujinaga et al., 1991, it is stated that the statistically significant increase in major visceral malformation was not seen in the table. Please see table 5 of the Annex to the CLH report. A statistically significant increase in situs inversus, cardiac anomalies and other visceral malformations was induced by dinitrogen oxide.
- In Mazze et al., 1984, it is questioned wether the increase in resorption was above normal variation. Although historical control were not provided, the control group in the study consisted in a higher number of rats (e.g. 160) giving weight to the concurrent control of the study. A clear statistically significant increase in resorptions was noted compared to the controls. It si also stated that the statistically significant increase in

minor skeletal anomalies was not found in publication. An increase in rudimentary lumbar rib, extra lumbar rib and total skeletal variants was reported in table 3 of the publication (table 23 of the CLH report). Nevertheless, we agree with the comment made to the remove cervical rib increase as the increase was only significant at the top dose level).

With regards to the comments on reproductive toxicity, please see response to comment number 3. In addition, we would like to highlight that although we agree with methodological shortcomings in the studies investigating effects on fertility and sexual function, this is not the case for the prenatal developmental toxicity studies where a large consistent database is available, including well-reported scientific published studies, dose-response assessment and mode of action analysis. Regards to fertility, although some limitation of the database is noted, the significant effects induced by dinitrogen oxide cannot be disregarded.

With regards to your comment on cobalamine deficiency, there are several studies investigating this potential mode of action for developmental toxicity by using folic acid to treat cobalamine deficiency:
- In Mazze et al. (1984), five rats from each group were killed immediately after exposure (day 10) and an additional five rats from each group were killed 24 hours later (day 11) to measure deoxyuridine (DU) suppression (bone marrow, embryonic cells). Immediately following exposure to 250,000 ppm dinitrogen oxide, DU suppression values (marker of cobalamine deficiency) in both maternal bone marrow and foetal cells were increased approximately 2.5-fold without concommittant increase in resorptions or malformations. Values returned toward normal in the next 24 hours.
- Keeling et al., 1986 observed that developmental effects were reduced in the combined dinitrogen oxide/folinic acid group but a statistically significant increase in major malformation was still observed compared to control.
- Mazze et al. (1988) exposed Sprague Dawley rats on day 8 of gestation to 50-75% N2O for 24 h alone or in combination with halothane or folic acid. Exposure to N2O alone resulted in a significantly increased number of resorptions and in major and minor skeletal abnormalities. Halothane administered in combination with N2O protected against these effects. In the contrary, folinic acid did not, suggesting that cobalamine deficiency may not, alone, explain the teratogenicity induced by dinitrogen oxide.

RAC's response

Thank you for the comment. The arguments have been taken into account in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
13.07.2022	Austria	<confidential>	Industry or trade association	8

Comment received

The studies submitted in the Proposal for Harmonised Classification and Labelling do not follow OECD test guidelines as suggested by the EU authority.
Moreover, the interpretation of the data is impaired due to substantial limitations in the animal studies. In the studies extremely high concentrations were used and could displace oxygen levels of animals. Hence, it is not evident if the mentioned effects were caused by N2O or hypoxia or other factors. Other potential modes of action (e.g. cobalamine deficiency) have not been discussed in the dossier. Besides that, most of the cited studies provide inconsistent results, the degree of detail of the studies vary highly and partly include small group sizes. Therefore, causal relation of N2O and fertility could not be proven.

In addition to that, only a very minor part of the studies reports effects on maternal toxicity. Hence, an assumed secondary non-specific consequence of other toxic effects cannot be excluded. Due to these factors, the classification of N_2O with respect to reproduction is scientifically not justified. Studies with human data did not provide reliable results since co-exposure to other influencing factors could not be excluded, e.g., contraceptives, smoking, anaesthetics, lifestyle etc.
Dossier Submitter's Response
Thank you for your comment, please see response to comment numbers 3 and 7. We disagree that the data are not consistant as most of the studies indicated potential effect on fertility or development. We agree that human data are not sufficient to propose category 1A for dinitrogen oxide.
RAC's response
Thank you for the comment. The arguments have been taken into account in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
11.07.2022	Netherlands		MemberState	9
Comment received				
Fertility: Several non-guideline and non-GLP studies with rats suggest possible effects on fertility. Similar effects were observed in different studies. However, reported testis effects were not always consistent. The information is supported with limited human information although no firm conclusions can be made based on any of the single studies because of study limitations. In addition, reliable or multiple generation studies indicating effects are lacking. Taken together there are signs fertility can be affected by dinitrogen oxide but there remains some uncertainty due to the study limitations and lacking generational fertility data. Therefore the NL-CA agrees that classification for effects on fertility in category 2 is most appropriate. The negative studies in mice cannot disregard positive effects in rats as its simply a different species. Although this implies there might be species differences, the NLCA is of the opinion this should not reduce the concern (without more information on species differences/MoA) as is suggested in the conclusion by the DS. Development: Dinitrogen oxide clearly causes teratogenicity in multiple studies when exposed to >500000 ppm dinitrogen oxide during a seemingly critical window of development. Unfortunately, the data presented does not include a good description of general toxicity of the dams. The description is either absent or limited to weight loss/drowsiness observations. This does make the potential of dinitrogen oxide to cause teratogenicity as a non-specific secondary effect uncertain. It is noted the weight losses described in some of the studies may partly be explained by resorptions and lower pup weights. However this may also be caused by drowsiness and lower food consumption (or something else). In a repeated dose toxicity study (Singh et al 2015) significant weight loss was also observed at 500000 ppm although the exposure duration was longer so it is difficult to compare. In addition, this repeated exposure study suffers from several limitations which makes a proper evaluation difficult. However, also adverse histopathological effects on the brain were observed at this dose level.				

In the end, it remains unclear if the teratogenicity might be a secondary effect to general toxicity. As the DS notes, according to the CLP criteria a secondary effect needs to be reasonably well demonstrated to reduce the significance of the effects. This is not the case and therefore would warrant classification in category 1B. However, the quality of the data demonstrating developmental effects without a good description on general/maternal toxicity might not be enough for classification in category 1B to start with. The reliability score of 2 attributed to such studies is also questionable since arguably the number of endpoints measured (disregard general toxicity) and reporting might be too limited for a score of 2. Further, consistent effects were mainly observed in rat studies with 23-24 hour exposure per day at often very high concentrations. In rat studies with intermittent exposure, the results are less consistent with both positive and negative studies. The relevance of the results of a 23-24 hour exposure for humans is questionable as workers are normally only exposed for 8 hours. Further, the very high exposures of for example 700.000 ppm could be considered as being above the limit dose stated in paragraph 3.7.2.5.7 of CLP as not relevant for classification. In conclusion, the NL-CA has a slight preference to classify dinitrogen in category 2 for effects on development rather than category 1B. A broad discussion at RAC will be required to come to a final conclusion on this endpoint.
Dossier Submitter's Response
Thank you for your comment and your support for classification of dinitrogen oxide in category 2 for effects on fertility. As regards to the developmental toxicity, we agree that the discussion wether the observed effects are specific or non-specific secondary effect will be key in the determination of the category for this endpoint. Please see response to comment number 3 explaining why we believe they are not non-specific secondary effects
RAC's response
Thank you for the comment. The arguments have been taken into account in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
11.07.2022	Germany		MemberState	10
Comment received				
Position: The DE CA agrees with the proposed classification for effects on sexual function and fertility (Repr. 2 (H360f)) based on adverse effects on oestrous cyclicity and female fertility observed in a short-term inhalation study in rats by Kugel et al 1990. In this study, 90 % of females exposed to 30 % of N2O for 8 h/d for 4 days entered constant pro-oestrus and only 50 % of treated females gave birth. The adverse effect on cyclicity was reversible; however, it required 3 weeks for normalisation. Other studies investigating female fertility in rats were not available. In mice, exposure to comparable doses of N2O did not lead to morphological changes in ovaries or to changes in number of oocytes, while other relevant endpoints were not investigated. Overall, in the light of clear but limited evidence of effects of N2O exposure on female reproduction and taking into account limitations of the non-GLP study (scored as Klimisch 2) supporting the FR proposal and limited evidence generated in the second species, classification as Repro. 2 (H360f) is considered appropriate. The DE CA as well supports the classification for adverse effects on development (Repr. 1B (H360D)) as proposed by the dossier submitter. The clear evidence of developmental				

toxicity supporting this proposal for classification includes death and impaired growth of developing organism and evidence of teratogenicity. In 17 studies suitable for a weight-of-evidence (WoE) analysis (Klimisch score < 3), the majority reports increases in resorptions (early and/or late), delayed development, increases in skeletal malformations (and variations), and visceral malformations. The most sensitive window of exposure with mild effects on pregnant dams is GD 8, when N2O is administered for 24 h at 500 000 ppm or up to 750 000 ppm. On the other hand, there were also two studies in mice where no effects on litter size, foetal growth and teratogenicity was observed. Overall, in spite of the potential species differences indicating that the rat might be a sensitive species towards developmental effects of N2O, the effects observed in rats are considered to be relevant to humans, as disruption of folate metabolism due to exposure to N2O was documented in both rats and in humans.

Comments:

a. Female reproductive cycle: Considering the mechanism-of-action of N2O via impaired folate metabolism (well-documented in humans and summarised in the STOT RE chapter of the CLH dossier), effects on ovary function and rate of implantations are expected (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4175124/). Regrettably, there is only one reliable study in exposed non-pregnant female rats (Kugel et al, 1990), that however demonstrated reduction in fertility and disruption of the oestrous cycle.

b. At the same time, it was unexpected that supplementation with folinic acid (performed by Mazze et al 1988) did not prevent the teratogenic effects of N2O.

c. Importantly, human evidence (https://pubmed.ncbi.nlm.nih.gov/6930434/) exists (included in the current CLH dossier in the Developmental toxicity sub-chapter), demonstrating an increase in spontaneous abortions in partners of male professionals exposed to dinitrogen oxide, which further supports the relevance of findings observed in rats to human reproduction.

d. In the current dataset the data on maternal toxicity are limited. From the available studies, the spectrum of the maternal general toxicity findings was dependent on the dose and duration of exposure and included drowsiness, reduced body weight, reduced food and water consumption, and in one study maternal death was reported. Overall, one may conclude that dams exposed to 500 000 ppm N2O for 24 h on GD 8 experienced mild sedation and at 750 000 ppm reduced food and water consumption and reduced motor coordination.

e. One critical point of the available database is a single-laboratory origin of most of the studies demonstrating developmental toxicity (7 out of 17 accepted for WoE studies). However, the scientific evidence of the data generated by these investigators is rather convincing, as experiments were carefully designed to control for the contribution of potential co-founders affecting pregnant dams, such as 24 h-long fasting or stress. These seven studies have a high rating in the WoE analysis, because all of them applied a similar protocol of exposure and demonstrated a high degree of reproducibility of the observed effects.

f. A question to the assessment of reliability of the open literature:

1) Please note that the publication by Rice et al, 1985 is given two different Klimisch scores (“4” on p. 13 and “2” on p.55).

2) Kugel et al, 1989 is assigned a Klimisch score 4, but the publication is used in the WoE

supporting the classification proposal.
Dossier Submitter's Response
Thank you for your comment and your support. We fully agree with your comments (a) to (e) that are in line with our present report. f.1. Thank you for spotting the inconsistency in Rice et al., 1985 rating. As the published study was not available, a rate of 4 was considered appropriate. Nevertheless, based on ECHA disseminated website robust summary, a score of 2 could also be considered. f.2. As only the abstract was available to us, Kugel et al., 1989 was assigned a klimisch score 4. However, as similar effects were reported in Kugel et al., 1990, we considered that the study could support the classification proposal.
RAC's response
Thank you for the comment. The arguments have been taken into account in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
08.07.2022	Sweden		MemberState	11
Comment received				
The Swedish CA supports classification of dinitrogen oxide (CAS No. 10024-97-2) as Repr. 1B H360Df. In general, SE CA agrees with the rationale for classification into the proposed hazard class and differentiations. However, the rationale/selection criteria of studies on developmental effects based on their assessed quality and (lack of) information on maternal toxicity is not clear to us. We note e.g. that results from studies assigned Klimish 4 and studies without information on maternal toxicity appear to be given similar weight as better quality-studies containing such information in the discussion. Studies lacking information on maternal information should not be given significant weight in the WoE determination. We believe that a higher transparency in this regard in the discussion would be beneficial for the comparison with the criteria and conclusion on classification for developmental toxicity.				
Dossier Submitter's Response				
Thank you for your comment. Regards to the absence of maternal toxicity data, there are some studies reporting maternal toxicity. As several studies were performed in the same laboratory, similar toxicity at same dose levels could be assumed. We consider that the description of available maternal toxicity allow to assess potential maternal toxic effect of the substance. In addition, please see response to comment 3 on maternal toxicity.				
RAC's response				
Thank you for the comment. The arguments have been taken into account in the RAC opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2022	United Kingdom	European Cylinder Makers Association	Industry or trade association	12
Comment received				
All of the animal studies have substantial limitations (non-GLP, not test guideline compliant as recommended by the European authority, single dose level, overtly high concentrations dosed, small group size) that make it difficult to interpret the data. It is further prudent to note that the overtly high concentrations used would have displaced				

oxygen levels, with no understanding of hypoxia compared to toxicity induced by exposure alone to dinitrogen oxide. The overtly high concentrations used in the animal studies exceeded the maximum recommended concentration as detailed in OECD TG for inhalation studies (20,000 ppm).
It is furthermore important to acknowledge that with only limited reporting of maternal toxicity, an assumed secondary non-specific consequence of other toxic effects cannot be excluded.
The human data provided by ANSES was only discussed briefly due to the lacking reliability of the results. These were retrospective studies which investigated the effect of dinitrogen oxide on midwifes and dental assistants, respectively. In the first study the exposure was estimated based on the average deliveries per month and in the second study the exposure was derived from the total scavenged dinitrogen oxide during dental therapies. The main bias in both studies was that a co-exposure to other factors influencing reproduction could not be excluded, e.g., smoking, contraceptives, exposure to other anesthetics, lifestyle, underlying disease etc. Thus, a clear causal relation cannot be established between dinitrogen oxide and fertility.
It should also be noted that there has been no discussion or consideration of other potential modes of action (hypoxia, cobalamin deficiency).
In conclusion, a classification of dinitrogen oxide neither in Category 1A or 1B (missing evidence in humans) nor in Category 2 with regard to toxicity to reproduction is not justified.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment ECMA_Submission_to_ECHA_July_2022_Rev_2.pdf

Dossier Submitter's Response

Thank you for your comment, please see response to comment numbers 3 and 7.

RAC's response

Thank you for the comment. The arguments have been taken into account in the RAC opinion.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	Austria	KAYSER Berndorf GmbH	Company-Manufacturer	13

Comment received

with limited reporting of the human volunteer studies (inadequate test article characterisation, methodology, acceptance / evaluation criteria) that are fulcrum to STOT SE classification, a reliability assessment cannot be made as detailed in Schneider et al (2009) Toxicology Letter, 189(20) pp 138-144).
For more specific information please see the attached position paper.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment ERM_KAYSER_Position_Paper.pdf

Dossier Submitter's Response

Thank you for your comment. We disagree that the data are limited.
Effects of nitrous oxide on cognitive function after single exposure were observed in several human volunteer studies. In these studies, exposure to nitrous oxide was well controlled. In addition, risk of Bias assessment, according to OHAT approach, was performed on these studies (NTP-OHAT, 2015). Concern for high potential of bias was only noted in Mahoney et al. (1988), Estrin et al. (1988) and Bruce and Bach (1976). In Bruce and Bach this was due to the high sensitive population studied. High risk of bias

was not identified for the other studies that are fulcrum to the proposal. In addition, clinical signs indicative of ataxia were also noted in animals exposed to nitrous oxide, supporting the proposal.
RAC's response
RAC agrees the reliability of the individual studies is difficult to assess. However, with similar findings, they can still be used in the weight of evidence to support classification.

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	Hungary	LISS Zrt.	Company-Manufacturer	14
Comment received				
: with limited reporting of the human volunteer studies (inadequate test article characterisation, methodology, acceptance / evaluation criteria) that are fulcrum to STOT SE classification, a reliability assessment cannot be made as detailed in Schneider et al (2009) Toxicology Letter, 189(20) pp 138-144). ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Comments on Proposal for Harmonised Classification and Labeling of Dinitrogen Oxide 7July2022.pdf				
Dossier Submitter's Response				
Thank you for your comment, see response to comment no. 13.				
RAC's response				
Thank you for your comment. See response to comment 13.				

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	Belgium	EIGA	Industry or trade association	15
Comment received				
The proposed harmonised classification on health effects is different from the classification used by our members. As required by REACH, dinitrogen oxide was registered by our members. A Chemical Safety Report and a classification were made at that time following a robust assessment of the available studies. The EIGA members have monitored the evolution of data published with respect to health and environmental 15argel with a view to reconsider the classification. It is our opinion that no research has been published that would change the classification of N2O. ERM, a consultancy firm sponsored by Kayser, reviewed in detail the literature study and annex mentioned on the webpage 'Registry of CLH intentions until outcome – dinitrogen oxide'. Report No : 0649151-Tox 1 from 7 july 2022 : "Proposal for Harmonised Classification and Labelling of Dinitrogen oxide : Position paper discussing reproductive and developmental CLH proposals." We have read this review via one of our members. EIGA will not copy the exact report with its comments but wishes to emphasize that our members support the comments contained within the report from ERM. ECHA note – An attachment was submitted with the comment above. Refer to public attachment N2O harmonized classification_EIGA comments.pdf				
Dossier Submitter's Response				
Thank you for your comment, see response to comment no. 13.				
RAC's response				
Thank you for your comment. See response to comment no. 13.				

Date	Country	Organisation	Type of Organisation	Comment number
13.07.2022	Austria	iSi GmbH	Company-Downstream user	16
Comment received				
The human volunteer studies cannot be used for a reliability assessment for the STOT SE classification due to their limiting reporting with inadequate test article characterization, methodology and acceptance criteria. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Position paper_Harmonised Classification and Labeling_iSi GmbH.zip				
Dossier Submitter's Response				
Thank you for your comment, see response to comment no. 13.				
RAC's response				
Thank you for your comment. See response to comment no. 13.				

Date	Country	Organisation	Type of Organisation	Comment number
13.07.2022	Austria	<confidential>	Industry or trade association	17
Comment received				
The human volunteer studies provide limiting reporting with insufficient test article characterization, methodology and evaluation criteria. Therefore, the studies do not prove as reliable.				
Dossier Submitter's Response				
Thank you for your comment, see response to comment no. 13.				
RAC's response				
Thank you for your comment. See response to comment no. 13.				

Date	Country	Organisation	Type of Organisation	Comment number
11.07.2022	Netherlands		MemberState	18
Comment received				
The NL-CA agrees a classification for STOT SE 3 is appropriate based on narcotic effects in both humans and animals. Although the effects could be considered for STOT SE 1 or 2 the effects observed in humans are considered transient and the animal studies do not meet the cutoff criteria as laid down in the CLP regulation.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you for your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
11.07.2022	Germany		MemberState	19
Comment received				
The DE CA agrees with the proposed classification for specific target organ toxicity –single exposure, STOT SE 3 (H336) based on narcotic effects. In human volunteer studies, impairment of cognitive function such as response latency, psychomotor speed, reaction time and attention were observed (Mahoney et al, 1988;				

Estrin et al, 1988; Fagan et al, 1994). Rapid recovery of psychomotor and cognitive functions was reported (Yajnik et al, 1996). Measurements of regional cerebral flow, regional cerebral metabolic rate and cerebral function revealed effects on nervous conduction in N2O exposed human volunteers (Gyulai et al, 1996; Williams et al, 1984). In rats, narcotic effects such as dose-related decreases in locomotor activity and decreases in correct response were observed at ≥ 300 000 ppm N2O exposure (Courtière et al, 1997; Dzoljic et al, 1994).

Based on the effects observed in humans and animals, STOT SE 3, H336 classification is warranted.

The DE CA agrees that STOT SE 1 or 2 17argely17cation is not warranted, because dose levels of N2O single-exposure causing significant non-lethal toxic effects in rats exceeded the guidance values for STOT SE 1 or 2 classification. Neuron vacuolation was observed in rats following ≥ 3 h exposure to ≥ 300 000 ppm N2O, which was reversible 3 h after exposure (Jevtovic-Todorovic et al, 2000, 2001, 2003 and 2005). After prolonged exposure (≥ 8 h) neuronal cell death was reported (Jevtovic-Todorovic et al, 2003).

Dossier Submitter's Response
Thank you for your comment and support.
RAC's response
Thank you for your comment.

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2022	United Kingdom	European Cylinder Makers Association	Industry or trade association	20

Comment received
Single exposure: from the available human volunteer studies provided in the CLH Annex, the limited reporting of the data does not provide a reliability assessment. With the limited reporting of methodology, lack test article characterisation retrospective application of the ToxRTool to assess reliability of the data (as detailed in Schneider et al (2009) Toxicology Letter, 189(20) pp 138-144) cannot be applied. ECHA note – An attachment was submitted with the comment above. Refer to public attachment ECMA_Submission_to_ECHA_July_2022_Rev_2.pdf
Dossier Submitter's Response
Thank you for your comment, see response to comment no. 13.
RAC's response
Thank you for your comment. See response to comment no. 13.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	Austria	KAYSER Berndorf GmbH	Company-Manufacturer	21

Comment received
The same failure and concerns as previously discussed regarding the animal data are also raised here. From the available animal data all of the studies had limitations, were not conducted to GLP or any relevant test guideline. Many of the studies were undertaken with a single dinitrogen oxide dose group, thereby unable to establish a concentration-

response relationship. Animal group numbers were generally below the recommended test guideline group numbers, thereby making statistical analysis (if undertaken) difficult to interpret. Where adversity was observed this occurred at concentrations that exceeded the maximum recommended concentration for repeat dose inhalation studies (20,000 ppm), without consideration of hypoxic effects. Collectively concerns are raised over the reliability of the data used to derive the classification.
With defined cut off values for STOT RE following inhalation, the CLP guidance dose/concentration values are provided below:
- Category 1: (rat [6 h], gas): C≤50 ppm;
- Category 2: (rat [6 h], gas) C>50, but <250 ppm)
The concentrations used in all the animal data considered for STOT RE exceed the cut-off values and therefore animal data should not be used to classify for either Category 1 or 2.
The human data considered as a weight of evidence approach for nervous system effects are taken from a collection of case study reports following misuse use of dinitrogen oxide do not consider co-exposure to other factors (e.g. other drugs which induce a state of stupor) which may suppress the nervous system. These data have not had reliability assessments undertaken, as previously detailed.
For more specific information please see the attached position paper.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment ERM_KAYSER_Position_Paper.pdf

Dossier Submitter's Response

Thank you for your comment. The classification proposal for STOT RE is largely based on human data. On this basis, there are no cut-off criteria for classification.

RAC's response

The effects observed in animals are indeed above the guidance value levels. However, they do support the findings in human case reports for which guidance values do not apply. Although the individual case report studies have unclear reliability, the large number with similar findings is unlikely an effect of chance and can therefore be used in a weight of evidence to support classification.

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	Hungary	LISS Zrt.	Company-Manufacturer	22

Comment received

The same failure and concerns as previously discussed regarding the animal data are also raised here. From the available animal data all of the studies had limitations, were not conducted to GLP or any relevant test guideline. Many of the studies were undertaken with a single dinitrogen oxide dose group, thereby unable to establish a concentration-response relationship. Animal group numbers were generally below the recommended test guideline group numbers, thereby making statistical analysis (if undertaken) difficult to interpret. Where adversity was observed this occurred at concentrations that exceeded the maximum recommended concentration for repeat dose inhalation studies (20,000 ppm), without consideration of hypoxic effects. Collectively concerns are raised over the reliability of the data used to derive the classification.
With defined cut off values for STOT RE following inhalation, the CLP guidance dose/concentration values are provided below:
- Category 1: (rat [6 h], gas): C≤50 ppm;
- Category 2: (rat [6 h], gas) C>50, but <250 ppm)
The concentrations used in all the animal data considered for STOT RE exceed the cut-off values and therefore animal data should not be used to classify for either Category 1 or 2.
The human data considered as a weight of evidence approach for nervous system effects

are taken from a collection of case study reports following misuse use of dinitrogen oxide do not consider co-exposure to other factors (e.g. other drugs which induce a state of stupor) which may suppress the nervous system. These data have not had reliability assessments undertaken, as previously detailed. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Comments on Proposal for Harmonised Classification and Labeling of Dinitrogen Oxide 7July2022.pdf
Dossier Submitter's Response
Thank you for your comment, see response to comment no. 21.
RAC's response
Thank you for your comment. See response to comment no. 21.

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	Belgium	EIGA	Industry or trade association	23
Comment received				
The proposed harmonised classification on health effects is different from the classification used by our members. As required by REACH, dinitrogen oxide was registered by our members. A Chemical Safety Report and a classification were made at that time following a robust assessment of the available studies. The EIGA members have monitored the evolution of data published with respect to health and environmental inpact with a view to reconsider the classification. It is our opinion that no research has been published that would change the classification of N2O. ERM, a consultancy firm sponsored by Kayser, reviewed in detail the literature study and annex mentioned on the webpage 'Registry of CLH intentions until outcome – dinitrogen oxide'. Report No : 0649151-Tox 1 from 7 july 2022 : "Proposal for Harmonised Classification and Labelling of Dinitrogen oxide : Position paper discussing reproductive and developmental CLH proposals." We have read this review via one of our members. EIGA will not copy the exact report with its comments but wishes to emphasize that our members support the comments contained within the report from ERM. ECHA note – An attachment was submitted with the comment above. Refer to public attachment N2O harmonized classification_EIGA comments.pdf				
Dossier Submitter's Response				
Thank you for your comment, see response to comment no. 21.				
RAC's response				
Thank you for your comment. See response to comment no. 21.				

Date	Country	Organisation	Type of Organisation	Comment number
13.07.2022	Austria	iSi GmbH	Company-Downstream user	24
Comment received				
The animal data considered for STOT RE should not be used to argue for a classification since it uses concentrations that exceed the cut-off values (- Category 1: (rat [6 h], gas): C≤50 ppm; - Category 2: (rat [6 h], gas) C>50, but <250 ppm), The human data meant to serve as a weight of evidence approach for nervous system				

effects refer to case study reports on misuse of N2O. They do not take into account co-exposure to other factors such as consumption of other drugs that suppress the nervous system. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Position paper_Harmonised Classification and Labeling_iSi GmbH.zip
Dossier Submitter's Response
Thank you for your comment, see response to comment no. 21.
RAC's response
Thank you for your comment. See response to comment no. 21.

Date	Country	Organisation	Type of Organisation	Comment number
13.07.2022	Austria	<confidential>	Industry or trade association	25
Comment received				
The concentrations used in all the animal exceed the cut-off values and should not be used to classify for either Category 1 or 2. The cited human data are taken from case study reports on misuse of dinitrogen oxide which do not take co-exposure to other factors (e.g. other drugs which induce a state of stupor) into account that may suppress the nervous system.				
Dossier Submitter's Response				
Thank you for your comment, see response to comment no. 21.				
RAC's response				
Thank you for your comment, see response to comment no. 21.				

Date	Country	Organisation	Type of Organisation	Comment number
11.07.2022	Netherlands		MemberState	26
Comment received				
Considering the numerous human data indicating neurological effects, both reversible and irreversible (at higher dose levels and considering the proposed mode of action), the NL-CA agrees with the proposed classification as STOT RE 1 (nervous system).				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you for your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
11.07.2022	Germany		MemberState	27
Comment received				
The DE CA agrees that classification for specific target organ toxicity –repeated exposure, STOT RE 1, (H372 (nervous system)) is warranted based on evidence from human data. Good quality information is available from poison centres and case reports. In particular, subacute combined degeneration of the spinal cord, myelopathy and generalised demyelinating polyneuropathy were observed. Follow-up studies reported persistent symptoms. Due to severe toxic effects observed in humans after repeated exposure, STOT RE 1 classification would be appropriate.				

Dossier Submitter's Response
Thank you for your comment and support.
RAC's response
Thank you for your comment.

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2022	United Kingdom	European Cylinder Makers Association	Industry or trade association	28
Comment received				
When considering STOT RE classification, solely based on animal data defined cut off values are provided in the CLP guidance. When administration via inhalation is the route of exposure the guidance provides the following: - Category 1: (rat [6 h], gas): C≤50 ppm; - Category 2: (rat [6 h], gas) C>50, but <250 ppm) With the same limitations as described above are also relevant for the animal studies used to conclude on STOT RE, with reliability of the data used to drive STOT RE deemed to be questionable. Adversity effects were observed at concentrations that exceeded the maximum recommended ECHA note – An attachment was submitted with the comment above. Refer to public attachment ECMA_Submission_to_ECHA_July_2022_Rev_2.pdf				
Dossier Submitter's Response				
Thank you for your comment, see response to comment no. 21.				
RAC's response				
Thank you for your comment. See response to comment no. 21.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Ozone Layer

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	United Kingdom		Individual	29
Comment received				
Continuing in the justification section of the CLH document it says "In addition, it has been known since 1970 that dinitrogen oxide depletes the stratospheric ozone. According to the world meteorological organization scientific assessment of ozone depletion (WMO, 2018), natural and anthropogenic emissions of dinitrogen oxide make a larger contribution to stratospheric ozone depletion than emissions of any of the individual ozone depleting long-lived halogenated source gases. Based on the current calculated ozone depletion potential-weighed emission (Ravishankara et al., 2009), JRC (2015) indicated that dinitrogen oxide is the largest of all ozone depleting substances. Therefore, action is required at community level and justify the need to classify dinitrogen oxide as hazardous to the ozone layer." Again I consider the wording to be misleading Those same reports go on to state that 60% of all N2O is naturally occurring and 40% done to man made. Of that 40% which is "man made", 3/4 is from agriculture (namely the emission from decomposing livestock manure). Of all the N2O released globally only 10% of N2O impacting the ozone layer is deliberately made by man, and the amount of ozone harm via N2O deliberately created by man has only a small impact on the ozone layer when compared to the amount of				

ozone harm caused by deliberately created halogenated molecules. The justification is flawed and appears deliberately to misinterpret the data presented in the reports it quotes
Dossier Submitter's Response
Thank you for your comment. We acknowledge that N2O is also naturally occurring. However, please note that impact assessment is not part of the CLP regulation.
RAC's response
Thank you for your comment. The orgin of pollution is not a part of the CLP Regulation.

Date	Country	Organisation	Type of Organisation	Comment number
14.07.2022	Belgium	EIGA	Industry or trade association	30
Comment received				
The literature regarding ozone depletion is also not new. The literature was considered by the registrants at the time of registration. The experts concluded the data to be conclusive but not sufficient for classification. ECHA note – An attachment was submitted with the comment above. Refer to public attachment N2O harmonized classification_EIGA comments.pdf				
Dossier Submitter's Response				
Thank you for your comment. There is no justification in your comment why the data were not considered sufficient for classification by the experts.				
RAC's response				
Thank you for your comment. RAC agrees with the DS that the provided data are relevant and reliable. An additional ODP value from Ravell *et al*. (2015) supports the conclusion by Ravishankara *et al*. (2009).				

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2022	United Kingdom	Health and Safety Executive	National Authority	31
Comment received				
Nitrous oxide (CAS 10024-97-2) The ozone depleting potential (ODP) of N2O used for comparison with the CLP criterion for classification as Hazardous to the Ozone was obtained from a journal article by Ravishankara et al. (2009). This CLP criterion is for an ODP greater or equal to the lowest ODP of the substances currently listed in Annex I to Regulation (EC) No 1005/2009 for the controlled substances under the Montreal Protocol (i.e., an ODP of 0.005 for chlorofluoroethane). Therefore, please could the CLH DS and RAC confirm that the calculation method used to derive the ODP of N2O is consistent with the methods used under the Montreal Protocol? We note that Scientific Assessments of Ozone Depletion prepared by a panel of experts are made available at least every 4 years to inform decisions about amendments and adjustments to the Montreal Protocol. The Scientific Assessment of Ozone Depletion: 2018 (WMO, 2018) is the latest in the series. ODPs are listed in Appendix A of this report with footnotes for a description of the methods used to derive each value and the reference. These ODPs are semi-empirical values or atmospheric model calculations evaluated based on the best available data and analysis methods. Methods therefore				

appear to differ between substances. Also due to consideration of recent experimental data, methods of analysis and/or assessment of recommendations, it is stated that ODPs in this Scientific Assessment may differ from official metrics for controlled substances currently written in the Montreal Protocol.

Although the latest Scientific Assessment of Ozone Depletion (WMO, 2018) supports that N2O significantly contributes to ozone depletion, no specific recommendations for the ODP value have been made. As noted by the CLH DS, the WMO (2018) states that there remains uncertainty about the long-term impact of N2O on ozone due to changes in stratospheric chemistry and dynamics caused by increasing greenhouse gas concentrations (Revell et al., 2015). On this basis, Revell et al. (2015) suggested that a single ODP value for N2O is of limited use.

Noting the above, a new Scientific Assessments of Ozone Depletion prepared by the WMO and/or UNEP is expected in 2022. If available in time, please could the CLH DS and RAC consider any relevant new information in the CLH assessment for N2O?

References:

WMO (World Meteorological Organization) (2018) Scientific Assessment of Ozone Depletion: 2018, Global Ozone Research and Monitoring. Project–Report No. 58, 588 pp., Geneva, Switzerland, 2018. https://www.esrl.noaa.gov/csd/assessments/ozone/2018/

Ravishankara, A.R., Daniel, J.S. and Portmann, R.W. (2009). Nitrous oxide (N2O): the dominant ozone-depleting substance emitted in the 21st century. Science, 326, pp.123-125.

Revell, L.E., Tummon, F., Salawitch, R.J., Stenke, A. and Peter, T. (2015). The changing ozone depletion potential of N2O in a future climate. Geophysical Research Letters, 42, pp. 10,047–10,055.

Dossier Submitter's Response

In the CLP criteria, there are no methods recommended for the ODP calculation and the DS has no information whether the methodology could differ from the one of the montreal protocol. As the 2022 report is still not available, we cannot include any new information.

RAC's response

RAC agrees with the DS response. RAC notes that the WMO 2022 report on ozone depleting substances was published online in Feb 2023. The consideration of N_2O as an ozone depleter does not appear to differ from the 2018 report and does not alter the assessment by RAC.

PUBLIC ATTACHMENTS
1. ECMA_Submission_to_ECHA_July_2022_Rev_2.pdf [Please refer to comment No. 12, 20, 28]
2. ERM_KAYSER_Position_Paper.pdf [Please refer to comment No. 3, 13, 21]
3. N2O harmonized classification_EIGA comments.pdf [Please refer to comment No. 6, 15, 23, 30]
4. Position paper_Harmonised Classification and Labeling_iSi GmbH.zip [Please refer to comment No. 7, 16, 24]

CONFIDENTIAL ATTACHMENTS
1. Comments on Proposal for Harmonised Classification and Labeling of Dinitrogen Oxide 7July2022.pdf [Please refer to comment No. 4, 14, 22]