Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

ozone

EC Number: 233-069-2
CAS Number: 10028-15-6

CLH-O-0000007279-64-01/F

Adopted

16 March 2023

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: ozone
EC number: 233-069-2
CAS number: 10028-15-6
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	France		MemberState	1
Comment received				
Proposals for classification STOT SE1, STOT SE3 et STOT RE1 have not been pee-reviewed by France.				
Dossier Submitter's Response				
Thank you for your comment. No response required.				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	France		MemberState	2
Comment received				
FR agrees with the conclusions presented in the CLH report. The 2-years NTP study (Boorman G. A. et al. (1995), Herbert R. A. et al. (1996)) conducted in B6C3F1 mice exposed to ozone shows an increase of alveolar/bronchiolar combined adenoma or carcinoma in males and females (statistically significant for females) and alveolar/bronchiolar carcinoma in females and alveolar/bronchiolar adenoma in males (statistically significant). In the NTP lifetime inhalation study conducted in B6C3F1 mice, an increase in alveolar/bronchiolar carcinoma in males (statistically significant) and alveolar/bronchiolar adenoma in females was reported (statistically significant). These findings are supported by the studies conducted in A/J mice that show development of lung tumors in both sexes (Witschi H. et al. (1999), Last et al. (1987),				

Hasset C. et al. (1985). FR agrees that due to high incidence of spontaneous tumors in controls, these studies cannot be used to support Carc. 1B classification.

We would like to know if you had considered a classification proposal as category 1B ? The effects demonstrated in the NTP studies, are, in our opinion, at the borderline of a Carc.1B.

Dossier Submitter's Response

Thank your for the comment. We agree that this is a borderline case.

RAC's response

RAC considered the effects are suitable for a Carc. 2 classification. The studies by Last and Witschi show a high frequency of spontaneous tumour incidences in the strain A/J mice, and therefore these studies are not regarded as supportive for Carc. 1B. In regards to the findings in the mice from the 2-year NTP study, only stat. sign. carcinogenic potential was found in females. In the lifetime NTP study in mice, the carcinogenicity potential was found in both male and female, however, the effects were found late in the study (particular in males). Taken all information into consideration, a classification in Carc. Cat. 2 is appropriate.

Date	Country	Organisation	Type of Organisation	Comment number
18.05.2022	United Kingdom	EuOTA	Company-Manufacturer	3

Comment received

10.9, Carcinogenicity, Table 22, page 61
With regard to the study by Last J. A. et al. (1987), this is a dual exposure to sodium chloride and ozone and as such should not be included in the overall weight of the evidence.
10.9, Carcinogenicity, Table 22, page 62
With regard to the study by Witschi H. et al. (1999), this study did not demonstrate a carcinogenic effect; the effect was non-statistically significant and was not reproducible in at 9 months and the effect was not seen in the reversibility group (Group C). Additionally there were no corresponding histopathological changes indicative of ozone toxicity.
10.9, Carcinogenicity, Table 22, page 64 and 65
With regard to the study by Hasset C. et al. (1985), this is a common tumour in mice with a high spontaneous background; the occurrence in controls should not be a reason for limited reliability.
No historical control data were provided to determine if the effects were within background.
10.9, Carcinogenicity, Table 22, page 66
With regard to the study by Kim M. Y. and Cho M. Y. (2009), it is unclear why this study has a reliability of 3 compared to the other studies.
Control histopathology is not reported; thus the interpretation of histopathological findings is not possible.
10.9, Carcinogenicity, Table 22, page 67 and 68
With regard to the NTP, Technical report series 440 (1994);
Neoplastic: females only; NOAEC 0.12 ppm.
Effects in males were within historical control values. Additionally, non-neoplastic histopathology at 0.12 ppm was related to the nasal tissue not lung.
Liver, hardarian gland and uterus effects are not associated with ozone exposure, as ozone is locally active and does not become systemic.
10.9, Carcinogenicity, Table 22, 2nd row, page 69
With regard to the NTP, Technical report series 440 (1994);

Incidence of alveolar/ bronchiolar adenoma or carcinoma was slightly increased compared to 2 year historical controls (15%) in females at 0.5 (16%, positive trend test) and 1 ppm (24%, statistically significant). Effects in males were within historical control values of 2 year study. Historical controls are not available for lifetime studies of this duration.
The most sensitive effect is the nasal histopathology - not the lung
10.9, Carcinogenicity, Table 22, page 71
With regard to the NTP, Technical report series 440 (1994), why is the reliability considered to be 2?
The NOAEC for neoplastic effects is 1.0 ppm.
10.9, Carcinogenicity, Table 22, page 72
With regard to the NTP, Technical report series 440 (1994) and Boorman G. A. et al. (1995), why is the reliability considered to be 2?
The NOAEC for neoplastic effects is 1.0 ppm.
10.9, Foot of table page 73
Please can you advise why the annotations are not included in the weight of the evidence?
10.9.1.1, Table 23, page 74
With regard to the study by Last et al. 1987), this is a co-exposure study and should not be included in the weight of the evidence. Additionally this table excludes negative results in the same study.
10.9.1.1, (3) Studies with B6C3F1 mice, page 83
With regard to the final sentence, "Due to the limited exposure time, this study cannot be considered as a fully reliable carcinogenicity study for this strain.", however, please note that it would appear that other limited exposures that did show a positive effect have been included.
10.9.1.1, (4) Studies with B6C3F1 mice and F344/N rats (a) 2-year studies, 2nd paragraph, page 83
Based on the results from the other studies, if this is a carcinogenic effect, it still should be observed. All animals were evaluated and stating 50% of animals died before termination should be compared with survival in other groups and when animals in this group died.
10.9.1.1, (4) Studies with B6C3F1 mice and F344/N rats (a) 2-year studies, 3rd paragraph, page 83
Please note that effects in males were within historical controls.
10.9.1.2, Summary of key studies in vivo, page 86
Please refer to the earlier comments in the genotoxicity section (e.g. pages 41-47). Ozone is not systemically bioavailable.
10.9.1.2, Summary regarding the relationship between ozone-mediated cytotoxicity and genotoxicity, page 87
In some studies the genotoxic effects are clearly associated with cytotoxicity.
We disagree with the oxidation products of ozone being included in this section. If you look into the decisions for hydrogen peroxide, the oxidation products are not included in the evaluation, therefore oxidation products for ozone should not be considered.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Ozone CLH-report commenting table_EuOTA_18.05.2022.pdf

Dossier Submitter's Response

10.9, Carcinogenicity, Table 22, page 61; 10.9.1.1, Table 23, page 74
Last J. A. et al. (1987): We disagree that co-exposure with sodium chloride would limit the interpretation of the study.

10.9, Carcinogenicity, Table 22, page 62
Witschi H. et al. (1999): the results of the study are reported correctly for each group. It is solely the interpretation of EuOTA, that the study did not demonstrate a carcinogenic

effect. We disagree, increased tumor incidences were observed and supported by histopathology.

10.9, Carcinogenicity, Table 22, page 64 and 65
Hasset C. et al. (1985): the study is given a reliability of 2 and it is clearly stated, that the reliability of results observed in exp. is 1. However, statistical analysis and interpretation of the neoplastic findings ist limited due to the high spontaneous tumour incidence. Historical control data was not avaibable. In any case and accordance with OECD the respective GD, higher weight would be given to the concurrent control (if this is within the HCD range).

10.9, Carcinogenicity, Table 22, page 66
Kim M. Y. and Cho M. Y. (2009): Rel. 3 was given due to an overall insufficient documentation of the study design and documentation of the study and its results (e.g. it remains unclear which organs were examined histopathologically). This information is provided in the biocides CAR – we suggest that this is made available to RAC.

10.9, Carcinogenicity, Table 22, page 67 and 68
NTP, Technical report series 440 (1994): the results are given correctly. Dose-respone was tested by Cochran-Armitage-test. The effects on liver, hardarian gland and uterus effects are regarded as systemic treatment related effects (resulting from ozone exposure).

10.9, Carcinogenicity, Table 22, 2nd row, page 69
NTP, Technical report series 440 (1994): HCD should not be used to negate positive findings in experiments, but to jugde the reliability of the concurrent control. The concurrent control is within the HCD. It is agreed, that there are local effects, but the neoplastic findings in the lung after exposure to ozone can not be dismissed.

10.9, Carcinogenicity, Table 22, page 71
NTP, Technical report series 440 (1994): Reliability of 2 is given due to the given major deviations (More than 50 % male rats in 2-year study died before study termination).

10.9, Foot of table page 73
Please refer to the text starting page 82 (bottom).
10.9.1.1, page 74-94; 10.9.1.2, page 95- 99:
The interpretation of the results as well as the weight if evidence approach regarding the classification and labelling of ozone are presented comprehensively by the DS. It is agreed, that the key questions is, if there is a systemic availability of ozone or ozone reactions products. Nevertheless, the observed carcinogenic effects were observed after ozone exposure and are therefore treatment related and relevant for classification.

RAC's response

RAC agrees with the DS jusifications. RAC notes that the whole database is suffering from quality issues, insufficient reporting and transparency. However, a clear occurrence of effects is reported in the many submitted studies available for the assessment by the DS.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	France		MemberState	4
Comment received				
FR agrees with the classification proposal Muta. 2, H341 as in vivo studies conducted on somatic cells clearly report ozone mutagenicity and genotoxicity in absence of cytotoxicity.				
Dossier Submitter's Response				
Thank you for your comment. No response required.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
18.05.2022	United Kingdom	EuOTA	Company-Manufacturer	5
Comment received				
10.8, Table 19, page 30 With reference to the study performed by Dillon D. et al. (1992), these effects were not dose related and occurred only at the lower concentrations. Higher concentrations, which were not identified as toxic, showed revertant levels similar to the concurrent control. Moreover, a certain variance occurred between the different experiments. Based on the results, ozone is not genotoxic in tester strains TA98, TA100, TA104 and TA1535 with and without metabolic activation. Ambiguous results were obtained with tester strain TA102.This strain is known for its sensitivity against oxidative mutagens. Furthermore, there were not historical controls available to validate the positive response. 10.8, Table 19, page 32 With reference to the study performed by Gooch P. C. et al. (1976), the finding in this study was increased chromatid deletions at only 36 hours in one study. The summary does not report that no chromosomal aberrations were reported at 12 hour and 36 hours in the same study. The study does not report that no changes were reported after exposure to ozone saturated solutions. This is not in our opinion an accurate reporting of the available data. 10.8, Table 19, page 33 With reference to the study performed by Guerrero et al. (1979), the relevance of these findings is questionable given that a lack of understanding behind the mechanism of the effects detectable in this assay that led to the deletion of the OECD 479 guideline in 2014. 10.8, Table 20, page 41 With reference to the study performed by Gooch P. C. et al. (1976), as discussed in the toxicokinetic section, ozone is not systemically bioavailable and would not reach the target cells; other compounds such as hydrogen peroxide were not classified as genotoxic even though there is the same local detoxification and only reaction products may be available systemically. 10.8, Table 20, page 42 With reference to the study performed by Kim M. Y. et al. (2002), as discussed in the toxicokinetic section, ozone is not systemically bioavailable and would not reach the spleen; other compounds such as hydrogen peroxide were not classified as genotoxic even though there is the same local detoxification and only reaction products may be available systemically. 10.8, Table 20, page 41 With reference to the study performed by Kim M. Y. et al. (2001), as discussed in the toxicokinetic section, ozone is not systemically bioavailable and would not reach the				

spleen; other compounds such as hydrogen peroxide were not classified as genotoxic even though there is the same local detoxification and only reaction products may be available systemically.
10.8, Table 20, page 43
With reference to the study performed by Haddad et al. (2009), as discussed in the toxicokinetic section, ozone is not systemically bioavailable, the results support the lack of ozone reaching the target tissue.
10.8, Table 20, page 44
With reference to the study performed by Cestonaro et al. (2017), as discussed in the toxicokinetic section, ozone is not systemically bioavailable, the results support the lack of ozone reaching the target tissue.
10.8, Table 20, page 46
With reference to the study performed by Guerrero et al. (1979), as discussed in the toxicokinetic section, ozone is not systemically bioavailable, the results support the lack of ozone reaching the target tissue.
10.8, Table 20, page 47
With reference to the study performed by Finkenwirth et al. (2013), as discussed in the toxicokinetic section, ozone is not systemically bioavailable, the results support the lack of ozone reaching the target tissue
10.8.1, (1) Mutagenicity studies in bacteria, last sentence, page 49
The last sentence is unnecessary and should be removed, "Given that mutagenicity was independent of S9-mix, ozone seems to act as a direct mutagen."
10.8.1, (3) Indicator tests in mammalian cells, page 51
We disagree with the conclusion. Dillon and Victorian both reported cytotoxicity. Action of ozone with TA102 are due to degradation product and not assignable to ozone. In the hydrogen peroxide assessment, similar genotoxicity findings were reported but were not considered in the overall classification because genotoxicity was attributable to degradation products and not hydrogen peroxide.
10.8.1, In Vivo, Page 51
As discussed in the toxicokinetic section, ozone is not systemically bioavailable, the negative results support the lack of ozone reaching the target tissue. Please reconsider this section.
10.8.1, (2) Mutagenic studies in vivo, (b) systemic effects, page 53
With reference to the study performed by Gooch et al. (1976), this study was negative for chromosome aberrations in bone marrow, leukocytes and spermatocytes.
10.8.1, (2) Mutagenic studies in vivo, (b) systemic effects, page 53
With reference to the study performed by Haddad et al. (2009), 3 ppm is very close to the LC50 value and the study did not report any clinical signs, toxicity, or mortality.
10.8.1, (2) Mutagenic studies in vivo, (b) systemic effects, page 53
Tice et al (1978) concluded - No increase in chromosome-type aberration levels, though a small increase in chromatid-aberration levels was seen. No increase in the levels of any chromosomal aberration type was seen in parallel direct bone-marrow preparations. Sister-chromatid exchange (SCE) levels and cell-replication rates, which were determined in the Chinese hamster peripheral lymphocyte cultures and also in bone-marrow samples from similarly treated mice, failed to show any ozone-induced changes.
10.8.1, (2) Mutagenic studies in vivo, (b) systemic effects, page 53
Zelac et al. (1971b) co-exposures, especially with radiation, should not be included in the weight of the evidence.
10.8.1, (2) Mutagenic studies in vivo, (b) systemic effects, page 55
Last paragraph, as discussed in the toxicokinetic section, ozone is not systemically bioavailable, the negative results support the lack of ozone reaching the target tissue.
10.8.1, (2) Mutagenic studies in vivo, (b) systemic effects, last paragraph, page 55
The last sentence should be deleted, "Therefore, the possibility that mutagenicity is solely triggered

by cytotoxicity as secondary effect should be neglected."
10.8.1, (3) Indicator tests in vivo, (a) local effects, page 55
Please revisit the details for the following study:-
Lee J., Madden M, Hatch G., Bottei G., Peden D., Adler K., Devlin R. (1997), DNA damage, Comet Assay (single-cell gel electrophoresis (SCGE) assay)
Similar to OECD 489
Ozone, gas, whole body 0.4, 1.0 ppm guinea pig for 2 hours
Guinea pig, Hartley strain
male
inhalation
N=3-5
Positive in guinea pig.
Within one hour after exposure bronchoalveolar cells were obtained from animals and analysed for DNA damage in the Comet assay. An increase of DNA damage was observed. But, in parallel an increase of cytotoxicity was observed.

10.8.1, (3) Indicator tests in vivo, (b) systemic effects, page 56
Please include the study by Lee et all (1997)
Lee J., Madden M, Hatch G., Bottei G., Peden D., Adler K., Devlin R.
1997
DNA damage
Comet Assay (single-cell gel electrophoresis
(SCGE) assay) Similar to OECD 489
0.4 ppm human for 2 hours
Negative -humans
No DNA single-strand breaks (SSB) in humans.
In the second study, which did not include an air control (negative control), the authors reported that exposure to ozone in subjects pretreated with placebo increased SSB compared with non-exercise air control.
10.8.2 Comparison with the CLP criteria, 1st Row of table, page 59
There is no indication the ozone reaches the systemic circulation (as summarized in the toxicokinetic section). The toxicity of ozone via inhalation is expected to be similar to that of hydrogen peroxide. The potential for ozone to reach the systemic circulation has been experimentally studied and even at extremely high dose concentrations. The potential for reactive products generated from local ozone lung reactions is similar to that as generated by hydrogen peroxide. Like hydrogen peroxide, ozone is metabolized locally and no systemic distribution of ozone is likely due to its reactivity. Like hydrogen peroxide, if ozone reached the systemic circulation (there is no indication that this occurs) it will be rapidly degraded.
In the body, hydrogen peroxide can also undergo copper and iron-catalysed reactions (the Haber-Weiss- and Fenton reactions) to produce highly reactive hydroxyl radicals, which are capable of oxidising biomolecules in close proximity. Hydroxyl radical formation therefore mediates the cellular toxicity of hydrogen peroxide through lipid peroxidation, enzyme inactivation and DNA damage.
https://echa.europa.eu/documents/10162/1e1085f8-5cd7-e878-d79b-3ccd18ed0996
https://echa.europa.eu/documents/10162/a6f76a0e-fe32-4121-9d9d-b06d9d5f6852
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/337708/Hydrogen_Peroxide_Toxicological_Overview_phe_v1.pdf
https://dm5migu4zj3pb.cloudfront.net/manuscripts/110000/110149/cache/110149.1-20201218131435-covered-e0fd13ba177f913fd3156f593ead4cfd.pdf
https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2021.EN-6806
https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_122.pdf

10.8.2 Comparison with the CLP criteria, 2nd Row of table, page 59

Gooch et al (1976) also evaluated spermatocytes in mice with no chromosome aberrations reported.
10.8.2 Comparison with the CLP criteria, 2nd Row of table, page 59
The references quotes are considered to be unreliable and should not be used in the weight of the evidence. Furthermore, no offspring defects have been reported in the toxicological database and no effects on fertility.
10.8.2, Comparison with the CLP criteria, 2nd Row of table, page 59
There are no conclusive studies demonstrating the genotoxicity of ozone to humans in vivo. In two available studies, ozone failed to induce DNA strand breaks in humans exposed to 0.4 ppm ozone for 2 hours (Lee et al. 1997) or SCEs in humans exposed to 0.5 ppm ozone for 2h (Guerrero et al. 1979).

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Ozone CLH-report commenting table_EuOTA_18.05.2022.pdf

Dossier Submitter's Response

There are a number of in-vitro assays showing the genotoxic potential of ozone. However it is agreed, that all studies have shortcommings in the reporting an/or study design and therefore have to be considered in a weight of evidence approach. Detailed responses:

- 10.8, Table 19, page 30
  Dillon D. et al. (1992): statistically significant increase of revertants were observed for diffentent strains. However, due to the reported shortcommings in study design and reporting the Dossier submitter considered the negative results for strains TA102 and TA104 of limited reliability (no positive controls and no cytotoxicity to confirm that ozone reached the target).
- 10.8, Table 19, page 32
  Gooch et al 1972: it is clearly reported that no dose-response was observed and that the study has numerours shortcommings.
- 10.8, Table 19, page 33
  Guerrero et al. (1979): it is agreed that the OECD TG 479 was deleted on 2nd April 2014 based on decision of the OECD council. However, the council also stated, that data previously generated from these deleted TGs can still be used in regulatory decisions.
  (https://www.oecd.org/env/ehs/testing/Draft%20Guidance%20Document%20on%20OECD%20Genetic%20Toxicology%20Test%20Guidelines.pdf) Here, a linear and statistically significant dose-related increase in SCEs per chromosome spread was reported in the study.

There are a number of in-vivo assay showing genotoxic effects after ozone exposure. It is certainly relevant whether there is a systemic availability of ozone or ozone reactions products. Regarding the toxicokinetik of ozone please refer also to our answer to comment 17.

- 10.8, Table 20, page 41
  Gooch P. C. et al. (1976): Please note that in other studies, systemic effects after ozone exposure could be demonstrated, but not in this study.
- 10.8, Table 20, page 42, 10.8, Table 20, page 41
  Kim M. Y. et al. (2001) & Kim M. Y. et al. (2002): Here, an increase in the number of chromosomal aberrations and MN vs. controls was observed, demonstrating potential for systemic effects after ozone exposure.
- 10.8, Table 20, page 43
  Haddad et al. (2009): Please note, the PCE/NCE + PCE ratio was reduced statistically significantly after treatment pointing to bone marrow toxicity due to ozone exposure.

- 10.8, Table 20, page 44
  Cestonaro et al. (2017): the systemic bioavailabilty was not questioned for this study. Please note, there are numerous limitations in data reporting versus guideline recommendations.
- 10.8, Table 20, page 46 & 10.8, Table 20, page 47
  Guerrero et al. (1979) & Finkenwirth et al. (2013): the systemic bioavailabilty was not questioned in this human volunteer study.

The interpretation of the results as well as the weight if evidence approach regarding the classification and labelling of ozone are presented comprehensively by the DS (10.8.1. page 49-59) regarding the vitro-assays and in-vivo assays als well as indicator assays and epidemiological findings. It is agreed that systemic availability of ozone or ozone reactions products is a relevant issue. Nevertheless, the genotoxic effects were observed after ozone exposure and are therefore clearly treatment related and relevant for classification.

The following studies are reported correctly:

- Lee J., Madden M, Hatch G., Bottei G., Peden D., Adler K., Devlin R. (1997), Comet assay in male guinea pigs, details are given on page 39.
  ON page 55 it is clearly stated, that "Cytotoxicity was indicated at 1 ppm by increased total protein and LDH content as well as changes in cell differentiation in bronchoalveolar lavage"
- Lee J., Madden M, Hatch G., Bottei G., Peden D., Adler K., Devlin R. (1997), Comet Assay in human volunteers, details are given on page 45. Only one dose was tested. There was no significant difference in DNA single strand breaks SSB in comparison to control (represented by change of DNA length) at this dose. A moderate increase in mean values was observed.

Your comments to section 10.8.2 Comparison with the CLP criteria are noted. It is agreed, that the data do not warrant a C&L for Muta. 1A. However, the DS is of the opinion that positive evidence for somatic cell mutagenicity and genotoxicity obtained from different in vivo studies warrants classification for Muta. Cat.2.

Please note, modifications to the CLH report are not foreseen at this stage.

RAC's response

RAC agrees with the DS responses and considers that the effects could also be caused by reaction products, which are to be expected to distribute more widely, or caused indirectly through a more complex adverse outcome pathway triggered by ozone. In any case, ozone remains the causative agent.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	France		MemberState	6

Comment received

Sexual function and fertility: Based on the data available, FR agrees that ozone does not meet the classification criteria for reproductive toxicity, for adverse effects on sexual function and fertility.

Development of the offspring: FR agrees that most of the studies available display deviations and cannot be used for classification. The study conducted by Bignami G. et al. (1994) similar to OECD 426 does not show effects on the offspring. The pregnancy duration is slightly increased in two highest dose groups, but as this effect is not seen in

other study, this is not considered for classification purpose. FR agrees that ozone does not meet the classification criteria for reproductive toxicity for adverse effects on development.
Dossier Submitter's Response
Thank you for your comment. No response required.
RAC's response
Noted.

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	France		MemberState	7
Comment received				
Based on the studies available, FR agrees with the conclusion that ozone is not an allergen and consequently a classification as respiratory sensitizer does not apply.				
Dossier Submitter's Response				
Thank you for your comment. No response required.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	France		MemberState	8
Comment received				
FR agrees with the proposed classification as that studies available report 50% mortality much below the concentration of 100ppm and agrees with the reasoning that, due to the absence of post-exposure observations, the toxicity may be underestimated. Regarding the ATE, we have noticed that the justification for the choice of this ATE is not mentioned, p14, when you compare the data with the classification criteria of CLP. Maybe you could add it ? We agree that according to Table 3.1.2, for ozone, the cATpE corresponding would be 10 ppm. Nevertheless, based on the data available, the LC50 is most likely lower than this value.				
Dossier Submitter's Response				
Thank you for the comment. Unfortunately, modifications to the CLH report are not foreseen at this stage.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	France		MemberState	9
Comment received				
FR agrees with the conclusion that the information available are not sufficient to conclude as for the skin corrosion/irritation potential of ozone. FR agrees that since ozone is a gas, classification as skin sensitizer does not apply.				

Dossier Submitter's Response
Thank you for your comment. No response required.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
19.05.2022	Netherlands		MemberState	10
Comment received				
Skin corrosion/irritation page 15-18 The DS has proposed 'no classification' because of a lack of skin irritation effects in various studies. However the NL-CA notices some uncertainties and considers that a discussion is needed. The available studies were not suitable to detect skin irritation effects but rather investigated changes in the upper dermis, such as oxidative state. Therefore, these studies can only be used as supportive information for effects in the skin, while they do not provide evidence for a lack of skin irritation potential, especially because the applied exposure levels (environmentally relevant) were low. In principle and in accordance with the CLP Guidance, strong oxidising properties provide a reason for concern for skin irritation / corrosion. In fact, the available information demonstrated the formation of reactive oxygen species in exposed skin, as well as depletion of antioxidants. There are no publicly available studies evaluating the irritation potential of ozone. However, considering the physical-chemical properties of ozone as a strong oxidising agent and the indications for induction of oxidative stress in the skin, classification for skin irritation should be discussed.				
Dossier Submitter's Response				
It is agreed that the physicochemical properties of ozone are giving rise to concern and further discussion is required whether in the absence of suitable toxicity data, this would be sufficient for classification.				
RAC's response				
RAC notes that there are indications of induction of oxidative stress in the skin but that the indications are considered insufficient to justify a classification for skin irritation. RAC agrees with the DS that, due to the absence of robust experimental evidence that can be used for classification purposes for this hazard class, no classification for skin irritation is warranted.				

Date	Country	Organisation	Type of Organisation	Comment number
18.05.2022	United Kingdom	EuOTA	Company-Manufacturer	11
Comment received				
10.4.3, page 18 The available human data can be used to support a Category 2 classification for both eye and skin irritation. The studies relied upon for inhalation irritation and other human studies considered in the dossier were of similar quality. The animal studies listed in Table 12 and the human studies in Table 13 in section 10.4 support a weight of the evidence approach that ozone is not corrosive to skin. Furthermore, the dossier states "human data on local skin effects				

may be obtained from existing data and corrosive reactions are typified by ulcers, bleeding and bloody scabs. In the human study submitted by the applicant, no signs of corrosion or erythema were reported.", which supports Category 2 for skin irritation. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Ozone CLH-report commenting table_EuOTA_18.05.2022.pdf
Dossier Submitter's Response
It is agreed, that available human data demonstrate some effects on the skin. However, the DS regards these studies in a weight of evidence approach as not applicable and/or sufficient to warrant classification for both eye and skin irritation according to the CLP criteria and they can only be used as supportive information.
RAC's response
RAC agrees with the DS that, due to the absence of robust experimental evidence that can be used for classification purposes for this hazard class, no classification for skin corrosion/irritation is warranted.

Date	Country	Organisation	Type of Organisation	Comment number
18.05.2022	Belgium	EurO3zon ivzw	Industry or trade association	12
Comment received				
Proposal not to classify for skin irritation/corrosion because it is not warranted from the available animal studies. All studies for skin corrosion/irritation have RI of 4.				
Dossier Submitter's Response				
No response required.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	France		MemberState	13
Comment received				
FR agrees with the conclusion that the information available is not sufficient to conclude as for the eye corrosion/irritation potential of ozone.				
Dossier Submitter's Response				
No response required.				
RAC's response				
RAC notes that although no data is available on corneal opacity, conjunctival redness or chemosis, taking into account the physico-chemical properties of ozone, and the indicative information from available studies demonstrating irritating effects in human eyes, as well as the inflammatory responses observed in animals, classification as Eye Irrit. 2; H319 could be considered.. However, RAC agrees with the DS's proposal and the comment by France and concludes that no classification for ozone for eye irritation/damage is warranted because the severity of effects observed at the concentrations tested are not sufficient to trigger classification for eye irritation/damage according to the CLP Regulation.				

Date	Country	Organisation	Type of Organisation	Comment number
19.05.2022	Netherlands		MemberState	14
Comment received				
Serious eye damage/eye irritation Page 18-22 The DS has proposed 'no classification' based on inconclusive data. However as the DS also suggests, H319 might be proposed based on weight of evidence. The NL-CA is of the opinion this should be discussed further. No well-established studies were identified for eye irritation and the concentrations used in the studies were rather low while they did indicate effects that lead to/are associated with irritation. It is noted that studies with higher concentrations levels that may produce irritation/corrosion effects are not available, also acknowledging that 4-hour LC50 values are already in the low range of 1-10 ppm. In principle and in accordance with the CLP Guidance, strong oxidising properties provide a reason for concern for eye irritation / corrosion and appropriate evidence must be provided in order to consider a no classification of substances with oxidising properties. Kleno and Wolkoff investigated blinking frequency and found only negligible effects of ozone comparable to control with regard to eye blinking. However, four out of eight subjects reported irritation from this substance (e.g. smarting, stinging, burning, and warming at the lower lid and/or the inferior part of the conjunctiva). Prabha et al. (2015), reported that at short-term exposure rates of 0.1–1.0 ppm, symptoms include headaches, nosebleeds, eye irritation, dry throat and respiratory irritation. These studies do not provide conclusive information but can be regarded as supportive for eye irritation potential. Thus, taking the physico-chemical properties into account and the indicative information from available studies suggesting irritating effects in human eyes as well as inflammatory responses observed in animals, classification as an eye irritant should be considered.				
Dossier Submitter's Response				
It is agreed that the physicochemical properties of ozone are giving rise to concern and further discussion is required whether, in combination with the limited human data and related information from animal studies, this would be sufficient for classification.				
RAC's response				
See response for comment 13.				

Date	Country	Organisation	Type of Organisation	Comment number
18.05.2022	United Kingdom	EuOTA	Company-Manufacturer	15
Comment received				
10.5.3, page 22 The available human data can be used to support a Category 2 classification for eye irritation. Section 10.5, animal studies support a lack of corrosivity. The concentrations used in the animals studies were supported by the toxicological database, which reports an LC50 in animals of 1.4-8.2 ppm. Testing above those levels is not supported based on humane animal standards (Table 11 in acute inhalation toxicity).				

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Ozone CLH-report commenting table_EuOTA_18.05.2022.pdf
Dossier Submitter's Response
Thank you for your comment. Please refer to response on comment no. 11 & 14.
RAC's response
See response for comment 13.

Date	Country	Organisation	Type of Organisation	Comment number
18.05.2022	Belgium	EurO3zon ivzw	Industry or trade association	16
Comment received				
Page 25 of the CLH report: Proposal not to classify for serious eye damage/irritation because it is not warranted from the available animal studies. For eye damage/irritation, there are 3 studies with RI of 4 and two with RI of 2. As for the human study with RI of 2, the following is indeed imporant: "Although these studies do demonstrate some effects to the eyes, these studies do not provide sufficient information to support classification for eye irritation as the studies are not directly applicable to this endpoint. According to Guidance on IR/CSA Section R.7.2.9.2 (6.0, 2017), the quality and relevance of existing human data studies should be critically reviewed. Reliable and relevant human data were not submitted."				
Dossier Submitter's Response				
Thank you for your comment. No response required.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
18.05.2022	United Kingdom	EuOTA	Company-Manufacturer	17
Comment received				
10.11.2 Comparison with the CLP criteria, 1st row, Impact on the nervous system, page 152 Impact on the nervous system: Ozone is not systemically bioavailable; therefore it has no potential to reach the target tissue of the neurological systems. Effects attributable to the oxidative products of ozone are not considered to be relevant to the classification of ozone as the oxidative products of hydrogen peroxide were not considered indicative or assignable to the toxicity of hydrogen peroxide. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Ozone CLH-report commenting table_EuOTA_18.05.2022.pdf				
Dossier Submitter's Response				
It is agreed, that the majority of ozone is expected to react with the tissue at the site of contact and that it is totally consumed almost immediately upon reactions with antioxidants and unsaturated fatty acids. These reactions generate the actual ozone messengers represented by either hydrogen peroxide as a fast acting compound or a variety of lipid oxidation products as late effectors.				

However that does not mean, that ozone is not bioavailable. It is agreed, that the effects could also be caused by reaction products, which are to be expected to distribute more widely, or caused indirectly through a more complex adverse outcome pathway (AOP) triggered by ozone. In any case, ozone remains the causative agent. Effects on the nervous system were reported in different studies after single exposure and hence a harmonised classification and labelling for specific target organ toxicity – single exposure is proposed: STOT SE 1, H370 – "Causes damage to organs (nervous system)".
RAC's response
RAC agrees with the DS response.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
18.05.2022	United Kingdom	EuOTA	Company-Manufacturer	18
Comment received				
10.12.1, Effects on the nervous system, page 194 Ozone is not systemically bioavailable. Additionally the effects in offspring were not reproducible in other studies and no neuropathology was reported in the NTP studies. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Ozone CLH-report commenting table_EuOTA_18.05.2022.pdf				
Dossier Submitter's Response				
It is agreed, that the majority of ozone is expected to react with the tissue at the site of contact, however that does not mean, that ozone is not bioavailable. Furthermore, reaction products might be expected to distribute more widely. Effects on the nervous system (morphological changes as well as behavioural changes) were reported in different studies for different ozone concentrations (pls. refer to table 45 & 46). Please note, in the NTP study with B6C3F1 mice as well as F344/N rats (lifetime inhalation study; Boorman G.A. et al. 1995) hypoactivity was reported particularly at 1 ppm.				
RAC's response				
RAC agrees with the DS response.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	France		MemberState	19
Comment received				
We agree with your proposal of classification for the environment. We have minor comments which should not impact the proposal. • p 215: we do not understand this sentence in the table: "3d-NOEC = 0.05mg/L TRO (0.006mg/L measured as TRO in algae solution" What is the difference between the first and the second TRO, is it nominal and measured, or total and equivalent to ozone? • The endpoint for Liropenaeus vannamei on p 219 (NOEC = 0.004 mg/L) in not present in the table on p 215 where higher endpoints are reported, could you please check? • P219 (11.7.1) it is indicated that acute studies are available for algae however no acute				

endpoint has been derived from these studies. Nevertheless, we agree that chronic endpoints are available and support that acute endpoints on algae should not change the classification. However, we note that according the Guidance on the Application of the CLP Criterai, the classification may be subject to further information becoming available.

Dossier Submitter's Response

Thank you for your comments

- The difference between the first and the second TRO is, that the first one is related to the concentration in seawater without algae while the second one was measured in the treatment with algae.
- The NOEC of 0.04 mg/L (not 0.004 mg/L) O_3 for *Litopenaeus vannamei* is already contained in the table.

Thank you for your agreement that acute algae endpoints would not change the acute classification for ozone.

RAC's response

M-factors are a legal requirement and are part of the classification. They should be applied irrespective of a subtances potential for use in a mixture.

Therefore, RAC is of opinion that setting of M-factors is a part of legal requirements for substances which are classified as aquatic acute/chronic in category 1 according to CLP Regulation and the setting of M-factors does not depend on the intended use of substance.

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	United Kingdom	Health and Safety Executive	National Authority	20

Comment received

Section 4.1 of the CLH Report states, in relation to human health classifications, that: 'ozone is generated from oxygen... as an in-situ substance' and 'As ozone is highly unstable and will not be placed on the market as part of a mixture, specific concentration limits (SCL) for the classification of mixtures are not necessary'. It is unclear to us whether a similar situation would also apply in the case of Aquatic M-factors - as they serve a very similar purpose to SCLs in mixture classification? We note the DS states that: 'It is at RACs discretion to include SCL for ozone in their Opinion if they see fit'. Could RAC please consider whether the setting of Aquatic M-factors is also applicable in this particular case, or not - and provide a reasoning. We note that there are also REACH-registered industrial uses of ozone, not just biocidal - however there will presumably be just one single set of harmonised classifications covering ozone as a substance. We do understand the reasoning behind the 'not being placed on the market as part of a mixture' and ambient air limit arguments - but wonder how far these are also applicable to all other uses of ozone and circumstances/downstream legislation in which a harmonised CLP classification may be utilised (e.g. waste, transport, hazardous site classifications)?

Dossier Submitter's Response

Thank you for your comment.
As this is a general issue and the comment is addressed primarily to the Risk Assessment Committee, we suppose that RAC will reponse to it.

Technically it is possible to derive M-factors for the environmental hazards from the available ecotoxicity data available for ozone. M-factors derived from this data are presented in the CLH-report.

RAC's response

See response to comment 19.

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2022	France		MemberState	21
Comment received				
No comments.				
Dossier Submitter's Response				
-				
RAC's response				
Noted.				

PUBLIC ATTACHMENTS
1. Ozone CLH-report commenting table_EuOTA_18.05.2022.pdf [Please refer to comment No. 3, 5, 11, 15, 17, 18]