Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

bixlozone (ISO); 2-(2,4-dichlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one

EC Number: -
CAS Number: 81777-95-9

CLH-O-0000007325-75-01/F

Adopted

8 June 2023

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON BIXLOZONE (ISO); 2-(2,4-DICHLOROBENZYL)-4,4-DIMETHYL-1,2-OXAZOLIDIN-3-ONE

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: bixlozone (ISO); 2-(2,4-dichlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one
EC number: -
CAS number: 81777-95-9
Dossier submitter: The Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	1
Comment received				
FMC submits the following comments for consideration by the Risk Assessment Committee (RAC) regarding the proposed Harmonised Classification and Labelling for bixlozone: FMC agrees based on the physical and chemical properties of bixlozone that classification for physiochemical properties and physical hazards it not required. FMC agrees that bixlozone does not meet the classification criteria for the following toxicology endpoints: acute oral, dermal and inhalation toxicity, skin and eye irritation, and skin sensitization, specific target organ toxicity – single and repeat exposure, germ cell mutagenicity, carcinogenicity, and reproductive and developmental toxicity. FMC agrees with the acute and chronic classifications proposed for bixlozone for environmental hazards and the endpoints upon which these are based.				
Dossier Submitter's Response				
Noted				
RAC's response				
Thank you for your comments. Support for proposed classification of bixlozone for environmental hazards is noted. RAC agrees.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	2

Comment received

FMC agrees with the DS that bixlozone does not meet the classification criteria for carcinogenicity.

FMC agrees that the low incidence of combined incidence of skin/subcutis fibroma and fibrosarcoma found in the male top dose (5000 ppm) group in the 2-year rat chronic study was not related to bixlozone treatment. The incidences of fibroma and fibrosarcoma across the dose groups did not show a dose response and were within the HCD range from the same laboratory. Therefore, the combined incidences represent background occurrences and are not related to treatment with bixlozone.

Similarly, the low combined incidence of thyroid follicular cell adenoma and carcinoma observed in high dose (5000 ppm) female rats in the 2-year chronic study are considered to be of spontaneous origin and do not represent a treatment-related effect based on the lack of hypertrophy or hyperplasia in the thyroid and incidences within the laboratory historical control range.

FMC agrees with the DS that there was no treatment-related increase in neoplasia in mice in the 18-month carcinogenicity study. Increases in histiocytic sarcoma, bronchiolo-alveolar adenoma/carcinoma or leiomyoma/leiomyosarcoma in cervix/uterus in high dose mice either lacked a dose-response and/or were within the laboratory historical control data.

Dossier Submitter's Response

Noted

RAC's response

Noted. See also response to comment 3.

Date	Country	Organisation	Type of Organisation	Comment number
03.08.2022	France		Member State	3

Comment received

Page 68 (rat): Historical Control Data (HCD) do not meet EFSA Administrative Guidance Requirements in terms of information provided (see excerpt below), only max. HCD are provided. In addition to the lack of information, the period of time largely exceeds 5 years (2009 to 2017 = 9 years). Given these two major issues, the HCD provided do not appear to be reliable or usable, it would be welcome if some clarification on that were provided.

For skin/subcutis-combined Fibroma/Fibrosarcoma, there is unclear dose-response (due to response in the mid-dose group), however incidence at the top-dose is 2-fold greater than in the concurrent control, and the low-dose has also greater incidence than the concurrent control, it is therefore difficult to consider these increases incidental. Could a discussion covering and weighing these findings be included too for completeness?

Page 68: For thyroid gland, there is monotonic increase of incidence of combined follicular cell adenoma/carcinoma (i.e., the value never changes direction) and the incidence is null in concurrent negative control. ADME do confirm that thyroid is well exposed and

hypertrophy were noted in the 90-day studies (rat, dog…). In this context and since HCD information is insufficient, one may deem that discarding the increase is not straightforward. Would it be possible to include a rationale discussing and weighing this increase in light of observations on thyroid in other studies?

Page 69 (mice): similar issues as page 68 (see above) are found with HCD. It is unclear too why combined adenoma and carcinoma, bronchio-alveolar HCD are "n.r." for "not reported" while HCD are available of these effects separated. Again, it would be welcome if clarification were provided on these issues with HCD.

Page 69 (mice): for low (250 ppm) and high (5000 ppm) doses, the corresponding incidences are greater than the HCD. Whilst no dose response is clearly found due to response in the mid-dose group, it is noted that at 5000 ppm the incidence is 2-fold greater than incidence observed in the concurrent control group (it is 1.5-fold at 250 ppm). This finding would probably be needed to be mentioned for completeness.

Excerpt from EFSA Administrative Guidance (EFSA Supporting publication 2019:EN-1612):
HCD are necessary to follow changes in the biology of the used test species and to differentiate the way to evaluate test results. HCD represent a summary of the observations made on the untreated or control groups from individual studies and a complete assessment of their relevance should be provided by the applicant in the dossier based on the criteria as set out in Commission Regulation (EU) No 283/2013:
- the incidences of effects for control animals in studies with the same design conducted by the same laboratory; summarised by species, sex, route of administration and vehicle. If study via diet, the diet should be mentioned with reference to the diet characteristics.
- the data for control animals compiled from the concurrent five-year period.
Therefore the following information should be provided:
- the mean, the median, the SD and range of incidences among studies of the effect,
- the number and the dates of studies summarised,
- the use of percentiles could be further considered for HCD of growth or survival (presented as curves),
- Single values (mean, median, SD and range) from those studies that fulfil criteria as set out in Commission Regulation (EU) No 283/2013.

Dossier Submitter's Response

Additional details on HCD according to the EFSA administrative GD might be requested from the applicant (for both rat and mouse findings). It should be noted that this dossier was submitted before the date of entry of this EFSA Administrative Guidance. The applicant confirmed that the HCD was provided from the same laboratory as where the study was performed and that the same strain was used in the studies.

In the rat study, to our opinion the increased incidence of combined fibroma and fibrosarcoma of the skin/subcutis in the 5000 ppm group in males do not have a dose response relationship among the dose groups (5%, 8%, 6% and 10%; not statistically significant), and the incidences do not exceed HCD 5 years prior or after the study period (HCD from same laboratory and using same rat strain). Therefore, the combined incidences represent background occurrences and are not considered related to treatment.

Considering the thyroid gland (incidence in males of 0%, 2%, 2% and 5%; not statistically significant), the DS remains of the opinion that no treatment related thyroid changes (e.g. lack of hypertrophy or hyperplasia in the thyroid) were seen and incidences

were within the laboratory ±5 years' historical control data and were also not corroborated by treatment-related thyroid changes in males. Therefore, these were considered of spontaneous origin and did not suggest a treatment-related carcinogenic effect.

In the mouse study, the slightly increased incidence of leiomyosarcoma and the combined incidence of leiomyoma/leiomyosarcoma in the cervix/uterus at the top dose are also not considered treatment-related (leiomyosarcoma 2%, 4%, 0%, 6% and combined incidence 4%, 6%, 2%, 8% (both not statistically significant). Although the combined incidence of leiomyoma/leiomyosarcoma is slightly above HCD (Table 6.5.2-7b), there is no clear dose-response relationship for either leiomyosarcoma or the combined leiomyoma/leiomyosarcoma. Therefore, this finding is not considered treatment-related.

RAC's response

RAC agrees that insufficient information was provided on the available HCDs. In several instances the number of provided studies is considered just adequate, in other inctances the number of available historical control studies is rather low (i.e. 2-5). Regarding the source of the HCDs the DS clarified that the studies came from the conducting laboratory and that they were conducted in the same strain as used in the studies with bixlozone. But important details are lacking, like the range and the median value for the rat HCDs, and the median value for the mouse HCDs. Upon RAC's request the DS clarified the actual study dates of both carcinogenicity studies (Rat study: Nov/2014 – Nov/2016; Mouse study: Nov/2014 – May/2016) and it can be concluded that the HCD from between 2009-2017 do not fulfil the ± 5 year requirement.

In line with the DS RAC is of the view that for skin/subcutis fibroma and fibroma/ fibrosarcoma combined there is lack of dose response. However, RAC agrees that for the fibrosarcoma alone, despite the low number of tumours, there seemed to be a dose response, for top dose males the incidence for this lesion just exceeded the HCD and there was no incidence in the concurrent control.

In female rats bengin and malign thyroid tumours were slightly increased with one follicular cell adenoma in the mid dose and 2 in the top dose as well as 1 follicular cell carcinoma in low and top dose each. These observations were below the provided upper range of the HCD. No hypertrophy or hyperplasia was seen in the thyroids in this study, however, non-neoplastic lesions were seen in the thyroids of rats and dogs in other studies. While in a 28 day dog study decreased colloid (mild) and nodular hyperplasia in C-cells (mild) were seen in one female of the top dose (1100 mg/kg bw/d) each (2 females / group) and in the 90 day dog study absolute and relative thyroid weight was increased in males at the two highest dose groups (300 & 750 mg/kg bw/d), there were no effects on the thyroid in the 1 year dog study (up to 500 mg/kg bw/d). In rats thyroid follicular cell hypertrophy (mild) was seen in males (3/10) & females (5/10) at the top dose of 505/351 (M/F) mg/kg bw/d. Overall these effects are rather inconsistent with regard to sex affected or type of effect induced and no such findings were seen in the studies of longest duration. It is also noted that these effects were seen at higher doses than applied in the rat carcinogenicity study, where thyroid tumours were seen. In conclusion, there were only few tumours in total, which clearly were below the provided HCDs, no related non-neoplastic lesions and the tumours were restricted to one sex. Overall this lowers the concern for a carcinogenic effect.

All incidences for these tumour types are within the upper range of the HCD, but there were issues with the HCD as discussed above.

RAC agrees that it should be possible to also provide HCD for bronicholo/alveolar adenomas and carcinomas combined. Reagrding the relevance of these lung tumours RAC is of the view that Bronchio-alveolar adenomas and carcinomas were seen in both, males and females of all dose groups, but no clear dose response was evident, also when adenomas and carcinomas were combined and a rather high incidence of carcinoma was seen in the concurrent controls of both males and females. Though tumours were seen in both sexes, bronchiolo-alveolar hyperplasia was only seen in males, without dose response (highest incidence in controls). The tumour incidences were clearly within the provided HCD range, which consisted of 7-11 control groups. Overall, the observed lung tumours are not considered supportive for a carcinogenic effect.

In top dose females 6 histiocytic sarcomas were reported, with 2 such tumours in the control group, but none in low and mid dose groups. In males only a single tumour was seen in the low dose group. The increase in top dose females was just above HCDs when percentage was considered, but within HCD when comparing to the absolute numbers (HCD: 2 – 7 (2 – 11.7%)). The HCD consisted of 11 studies, which is considered adequate and the HCD range, but not the median value was provided. In this case the median value would be helpful to interpret the meaning of the 6 histiocytic sarcomas in top dose females on the upper edge of the HCD range. Overall it can be concluded that the total lack of such tumours in low and mid dose females and the absence of tumours in mid and top dose males reduce the relevance of the finding.

In cervix/uteri of female mice leiomyomas and leiomyosarcoma were reported. There was a lack of dose response for leiomyoma, as there was a single incidence in each group including control. Also for leiomyosarcoma no dose response was obvious, but in the top dose the incidence was at the upper range of the HCD and when leiomyoma and leiomyosarcoma are combined the incidence in the top dose as well as in the low dose slightly exceeds the HCDs. Based on the available HCD it might be concluded that leiomyomas and leiomyosarcoma are rare tumours, but the HCDs consisted of 2-5 studies only. The presence of a single leiomyoma in each group, including the control could be an indication of slightly higher incidences of this tumours type in the animals on the present study compared to the HCDs. The lack of dose response reduces the concern.

Overall, the observed tumours were seen at rather low incidences, only partly exceeding the HCDs, though these HCDs have uncertainties, they often lack dose response, are seen in one species and mostly only in one sex. Overall RAC is of the view that these findings are not supportive for a classification.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	4
Comment received				
FMC agrees that bixlozone does not meet the classification criteria for genotoxicity/germ cell mutagenicity based on the results from a battery of in vitro and in vivo guideline genotoxicity studies. Bixlozone did not induce gene mutations in two in vitro assays and was negative for chromosome aberrations (numerical and structural) in an in vivo micronucleus assay.				
Dossier Submitter's Response				
Noted				

RAC's response
Noted. For exactness, the in vitro assay in mammalian cells for gene mutations was partly positive, i.e. when S9 mix was added, though only in the presence of cytotoxicity. RAC agrees however that there is no evidence supporting a classification as germ cell mutagen.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	5
Comment received				
FMC agrees that no classification is warranted for reproductive or developmental toxicity for bixlozone. There were no adverse effects observed in the rat two generation reproduction study and rabbit prenatal developmental toxicity study. In the rat developmental toxicity study, a slightly increased incidence of 14th rudimentary ribs and mal-aligned sternebrae was seen at high dose level. However, these are common variations observed only at high dose, occurred in the presence of significant maternal toxicity and showed no dose response or statistical significance when compared to the controls. The incidences were within historical control data range. Therefore, these variations are not considered to be of toxicological significance. No classification is warranted for reproductive or developmental toxicity for bixlozone.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted. More details on the historical control data would be useful to help interpret the findings. RAC is not of the view that the observed effects in dams of the main rat study can be described as "significant maternal toxicity".				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	6
Comment received				
FMC agrees bixlozone does not meet the criteria for classification for acute oral, dermal or inhalation toxicity based on the available data. • Acute oral LD50 >2000 mg/kg bw • Acute dermal LD50 >2000 mg/kg bw • Acute inhalation LC50 > 2.11 mg/L				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	7
Comment received				
FMC agrees bixlozone does not meet the criteria for classification for skin corrosion/ irritation based on the available data.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	8
Comment received				
FMC agrees bixlozone does not meet the criteria for classification for serious eye damage/irritation based on the available data.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted. See also response to comment 9.				

Date	Country	Organisation	Type of Organisation	Comment number
03.08.2022	France		Member State	9
Comment received				
Ocular irritation/serious eye damage endpoint: two tests were conducted, EpiOcular based on RhCE (OECD 492) and Draize rabbit eye test (OECD 405). The two show conflicting results and no rationale has been provided on how to rule out this endpoint. EpiOcular test gave a clear positive result (cell viability 19% only) and should have been followed by another test like BCOP according to OECD Guidance Document 263. Rabbit eye test show scores in favour of a negative results. It would probably be beneficial to include a rationale, RMS seems to give precedence to the rabbit test but without justification. EpiOcular gave a cell viability far below the threshold of 60% i.e., is clearly positive and Draize test is well known to have too high variability (ref. OECD GD 263) and therefore sound justification would be welcome. Indeed, by following a bottom-up approach (OECD GD 263) one may consider that the EpiOcular is positive (i.e., NOT No Category, either Cat. 1 or Cat. 2) and based on scores form the Draize test the final overall outcome is Cat. 2.				
Dossier Submitter's Response				
Noted. If requested by RAC, the DS could provide a further rationale for giving the precedence to the existing *in vivo* Draize rabbit eye test (OECD 405). This study was available as the applicant performed this study for the purpose of global submission (i.e. not all countries accept *in vitro* assays in lieu of *in vivo* data).				

RAC's response
The results of the two studies are rather clear, with a completely negative animal study and clearly positive in vitro study (the cut-off for cell viability of 60 was clearly underrun after bixlozone treatment to 19.6%). In the following RAC's weight of evidence analysis of the relevance of the two studies is provided. The OECD 492 guideline specifies for the applied test system (EpiOcular™) that no classification is indicated for substances that reduce cell viability to levels > 60%, whereas for a substance that reduces cell viability to levels below 60%, no distinction can be made between category 1 and 2 and no prediction can be made in isolation. In this respect, it is relevant to note that OECD 492 has a rather high rate of false positive results. For EpiOcular™ it is as high as 37% (based on 55 chemicals), when compared to reference in vivo rabbit eye test data (OECD 405), see OECD 429, para 14. According to OECD 492 and OECD GD 263 a positive result of an OECD 492 test requires further testing with (an)other in vitro test method(s), or as a last option in rabbits (OECD 405). It is not known to RAC why in this case an in vitro test was carried out after a reliable in vivo study was available, however, the above described step-wise procedure would normally conclude with the result of a reliable in vivo study according to OECD 405. RAC notes that the in vivo study (OECD 405) has strengths as compared to the in vitro studies (i.e. it reflects all possible modes of action, it formed the basis for the classification system, reversibility/persistence of effects can be directly observed), but also certain weaknesses, including that identification of category 1 substances based on effects in a single eye is related to uncertainty, allocation of the scores might be subjective, uncertainties regarding the actual exposure duration being influenced by species differences in relation to the type of test material or possibility that mechanical damage is induced if the test material is solid (see OECD 263). It is however, noted that the majority of these weaknesses is not relevant in the present in vivo study, as the test material was applied diluted (not as a solid) and as the only type of effect seen was conjunctival redness and chemosis, which was only observed at 1h post-installation and no effects at all were seen after 24h or later (identification of Catergory 1 is not relevant, "no effect" is less prone to subjectivity than grading of the degree of an effect). On that basis it appears that the in vivo results is reliable and based on the knowledge that false positive results are often achieved with the applied in vitro test, no classification for eye irritation is supported.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	10
Comment received				
FMC agrees bixlozone is not a dermal sensitizer and does not meet the criteria for classification for skin sensitization.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	11
Comment received				
FMC agrees with the proposal of no classification of STOT-SE for bixlozone.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	12
Comment received				
FMC agrees with the proposal of no classification of STOT-RE for bixlozone.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	13
Comment received				
FMC agrees with the acute and chronic classifications proposed for bixlozone for environmental hazards and the endpoints upon which these are based.				
Dossier Submitter's Response				
Noted				
RAC's response				
Thank you for your comment. Support for proposed classification of bixlozone for environmental hazards is noted. RAC agrees.				

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2022	Germany		MemberState	14
Comment received				
We thank the RMS for the detailed assessment. We support the conclusion to classify bixlozone as Aquatic Acute 1, with an M-factor of 1, and as Aquatic Chronic 1, with an M-factor of 10. However, we have some additional comments: 2.8.2.1.1: Ready biodegradability				

We agree that bixlozone is not readily biodegradable and that this study is a key study on the rapid degradability potential of bixlozone. But, could you please for reasons of clarity add the conclusion that bixlozone is not rapidly degradable. 2.8.2.2.1: Aerobic mineralisation We agree that the study is a supportive study to conclude on the rapid degradability potential of bixlozone. But, please, add the conclusion that bixlozone is not rapidly degradable. Aerobic metabolism in water/sediment systems We agree, that the study is a supportive study to conclude on the rapid degradability potential of bixlozone. But, please, add the conclusion that bixlozone is not rapidly degradable.
Dossier Submitter's Response
Noted. We agree that bixlozone is not rapidly degradable. This can be added to the conclusion.
RAC's response
Thank you for your comment. Support for proposed classification of bixlozone for environmental hazards is noted. RAC agrees.

Date	Country	Organisation	Type of Organisation	Comment number
03.08.2022	France		MemberState	15
Comment received				
Page 152: FR agrees with the classification proposal for environmental hazards and with the proposed acute and chronic M factor.				
Dossier Submitter's Response				
Noted				
RAC's response				
Thank you for your comment. Support for proposed classification of bixlozone for environmental hazards is noted. RAC agrees.				

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2022	United States of America	FMC	Company-Manufacturer	16
Comment received				
FMC agrees based on the physical and chemical properties of bixlozone that classification for physiochemical properties and physical hazards it not required.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				