Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

clofentezine (ISO); 3,6-bis(*o*-chlorophenyl)-1,2,4,5-tetrazine

EC Number: 277-728-2

CAS Number: 74115-24-5

CLH-O-0000006816-65-01/F

Adopted

11 June 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CLOFENTEZINE (ISO); 3,6-BIS(O-CHLOROPHENYL)-1,2,4,5-TETRAZINE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public 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 public 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 public 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.

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Substance name: clofentezine (ISO); 3,6-bis(o-chlorophenyl)-1,2,4,5-tetrazine
EC number: 277-728-2
CAS number: 74115-24-5
Dossier submitter: Spain

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Germany		MemberState	1
Comment received				
This CLH Report is based on a Renewal Assessment Report (RAR) prepared in the context of PPP Regulation in accordance with Commission Regulation (EC) No. 844/2012. It is noted that the RAR was submitted to public consultation (29 Dec 2018) and was already commented by DE-CA at this occasion. The review by DE provided here focusses on the proposed classification as Carc. 2 and the physical hazards.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
18.09.2019	Netherlands		MemberState	2
Comment received				
Thank you for the extensive evaluation and detailed presentation of the carcinogenicity endpoint. The following is noted with respect to the thyroid follicular tumours: - Only observed in rats and not in mice; non-genotoxicity; - In high dose male group (400 ppm), there was a slight (not statistically significant) increase in the total number of follicular cell tumours (adenoma and carcinoma), i.e. 8/50 vs 2/50 in controls; there was a significant positive dose-related trend in thyroid follicular cell tumours; non-neoplastic changes in the thyroid included agglomeration of colloid and slight increase in follicular cell hyperplasia; - No historical control data were presented for the thyroid follicular tumours, except for				

results of one single, concurrently performed, study. The incidence of the high dose clofentezine group was marginally higher than the incidence in control group from this concurrently run study (8/50 vs. 6/49);
- Mechanistic studies pointed towards a mechanism via induction of UDP-glucuronyltransferase.
Overall, we consider that a mode of action via induction of UDP-glucuronyltransferase is sufficiently demonstrated and therefore it is agreed that the slightly increased incidence of thyroid tumours as observed in the rat carcinogenicity study is not relevant for humans.

With respect to the liver tumours:
- Only observed in mice and not in rats; non-genotoxicity
- a significant positive trend in benign hepatic tumours in females; a slight (not statistically significant) increase in benign liver tumours in the high dose female groups (7/52 vs. 4/54 in controls); incidence at high dose group (7/52; 13.5%) is outside historical control range (0-7.7%);
- accompanied by a significant positive trend in combined benign+malignant hepatic tumours in females; a statistically significant increase in combined benign+malignant liver tumours in the high dose female groups;
- mechanistic studies do not present sufficient evidence for a specific mode of action.
Overall, the NL CA consider that the liver tumours cannot be fully discarded and a category 2 classification is supported.

Dossier Submitter's Response

The overall assessment of carcinogenicity included in the CLH Report is in line with NL CA comments.
As it is indicated in the NL CA comments, the weight of the evidence indicates that thyroid tumours are not relevant for humans.
With respect to the carcinogenic potential in the liver, the increase in the incidence of adenomas at the highest dose in female mice was not statistically significant but a significant positive trend after trend analysis was observed. Also, the increase was above the historical control, although only slightly. The combined analysis of benign and malignant hepatic tumours in females of the highest dose was also significant after pairwise comparison and showed a positive trend after trend analysis. Besides, although the higher incidence of hepatocellular adenomas exceeded those of the controls only at the highest dose level tested, it seems that tumours were not related to excessive toxicity. The mortality observed in CD-1 female mice at this dose was not considered due to treatment with clofentezine and rather a common age-related condition in CD-1 mice with a tendency of development of amyloidosis, which is a frequent cause of death in CD-1 mice. Mechanistic data provided were insufficient to dismiss elements of uncertainty in the liver tumour profile.
Consequently, the Spanish CA considers that the overall available evidence is deemed to match the criteria for classification as Carc. 2; H351 - Suspected of causing cancer.

RAC's response

RAC agrees that the thyroid follicular tumours can be explaned by the UDGPT MoA.
Regarding the liver tumours, RAC also recognises that some events/steps of the proposed CAR MoA are missing. In particular studies in human hepatocytes, in CarKO/PxrKO mice hepatocytes and in wild type hepatocytes are missing. However, the incidences of the adenomas are only observed in one sex, one species and only in the highest dose tested and were not statistically significant in pairwise analysis. Many of the key events and in the associative events are described and are justified and indicate a CAR MoA may be responsible for the observed liver adenomas. RAC therefore concluded that no classification for carcinogenicity is warranted.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CLOFENTEZINE (ISO); 3,6-BIS(O-CHLOROPHENYL)-1,2,4,5-TETRAZINE

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Germany		MemberState	3
Comment received				
Thyroid and hepatocellular neoplasia was observed in rats and mice. The DE-CA agrees with the DS that despite extensive and elaborate MoA analysis, the mechanistic data is not complete and thus findings cannot be dismissed. In view of the unlikely relevance of the MoA postulated for the thyroid tumors, likely lack of genotoxicity and the substantial data on the MoA for liver tumors, Cat. 1B however is clearly not justified. The proposal for Carc. 2 is considered appropriate and the remaining gaps in the MoA analysis should be closed.				
Dossier Submitter's Response				
The Spanish CA agrees with the comments.				
RAC's response				
RAC agrees that the thyroid follicular tumours can be explaned by the UDGPT MoA. Regarding the liver tumours, RAC also recognises that some events/steps of the proposed CAR MoA are missing. In particular studies in human hepatocytes, in CarKO/PxrKO mice hepatocytes and in wild type hepatocytes are missing. However, the incidences of the adenomas are only observed in one sex, one species and only in the highest dose tested and were not statistically significant in pairwise analysis. Many of the key events and in the associative events are described and are justified and indicate a CAR MoA may be responsible for the observed liver adenomas. RAC therefore has concluded that no classification for carcinogenicity is warranted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	4
Comment received				
Carcinogenicity assessment for clofentezine: A full data package is available for clofentezine. Furthermore, 2-year chronic/carcinogenicity studies in rat and mouse are available. In the carcinogenicity studies thyroid follicular cell tumours in male rats and hepatocellular adenomas in female mice were recorded. In the position paper included in the attachment the human relevance of both tumours is discussed (see file ADAMA_001 in the attachment). Based on the conducted mode of action work, the thyroid follicular cell tumours in male rats were considered non-human relevant and thus do not warrant a cancer classification. The slight increase in benign liver tumours seen in females only in the mouse carcinogenicity study are likely to be via a phenobarbital-like mode of action, and therefore can be considered non-relevant for humans. The absence of a clear dose response (dose levels were spaced 10-fold apart), the malignant hepatocellular tumours falling within the historical control data, only an increase in benign hepatocellular tumours, which were slightly above the HCD and these tumours were only observed in one species (mice) and one sex (females). Furthermore, control animals were at the higher end of the HCD range and the study duration was longer (104 weeks) compared to contemporary studies (78 weeks), which reduces the concerns regarding these tumours. Based on the available data it is the applicant's position that a cancer classification for clofentezine is not warranted. Further work to strengthen this conclusion for mice will be conducted shortly, including an in vitro comparative hepatocyte proliferation study (mouse, human) to investigate enzyme induction, cell proliferation and species differences, and a short-term repeat dose				

mouse toxicity study to investigate enzyme activity and hepatocyte proliferation, results of this work are expected the first half of 2020. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public attachment to ADAMA comments on clofentezine CLH report.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip
Dossier Submitter's Response
The Spanish CA agrees that thyroid follicular cell tumours in male rats are not relevant for humans. However, the increase in benign liver tumours observed in mice is considered relevant following the reasoning included in the chapter 10.9 of the CLH report (see also comment no. 2).
RAC's response
RAC agrees that the thyroid follicular tumours can be explaned by the UDGPT MoA. Regarding the liver tumours, RAC also recognises that some events/steps of the proposed CAR MoA are missing. In particular studies in human hepatocytes, in CarKO/PxrKO mice hepatocytes and in wild type hepatocytes are missing. However, the incidences of the adenomas are only observed in one sex, one species and only in the highest dose tested and were not statistically significant in pairwise analysis. Many of the key events and in the associative events are described and are justified and indicate a CAR MoA may be responsible for the observed liver adenomas. RAC therefore has concluded that no classification for Carcinogenicity is warranted.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	5
Comment received				
No comment ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public attachment to ADAMA comments on clofentezine CLH report.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	6
Comment received				
No comment ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public attachment to ADAMA comments on clofentezine CLH report.zip				

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip
Dossier Submitter's Response
Noted.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	7
Comment received				
No comment ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public attachment to ADAMA comments on clofentezine CLH report.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	8
Comment received				
No comment ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public attachment to ADAMA comments on clofentezine CLH report.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	9
Comment received				
No comment				

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Dossier Submitter's Response
Noted.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	10
Comment received				
No comment ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public attachment to ADAMA comments on clofentezine CLH report.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip				
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
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	11
Comment received				
No comment ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public attachment to ADAMA comments on clofentezine CLH report.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip				
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
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	12

Comment received

The applicant agrees with that no specific target organ toxicity – repeat exposure (STOT-RE) classification is warranted for clofentezine. As discussed in the CLH dossier in only one study (90-day rat study) effects on the liver were noted within the concentration range for a STOT-RE classification. The findings at 4000 ppm (corresponding to 265 and 292 mg/kg bw/day for males and females respectively) were above the trigger level for a STOT-RE2 classification.
At 400 ppm (corresponding to 26.2 and 29.3 mg/kg bw/day for males and females respectively), cholesterol levels were slightly increased, which was fully reversible after cessation of treatment. There was no effect on AST levels at 12 weeks of treatment for males and females, ALT was slightly decreased at week 12 for males but not females. No change in AP was recorded. LDH was increased in week 4 for males, week 8 for females, but was not different to controls at 12 weeks of treatment.
At 400 ppm, absolute liver weight was slightly increased after 13 weeks of treatment (10.9% (M) and 12.7% (F), relative liver weight 13.4% (M) and 9.2% (F). However, this was fully reversible 6 weeks after cessation of treatment and considered an adaptive response.
Centrilobular hepatocyte enlargement was observed amongst male at doses ≥ 400 ppm but not in females which was reversible after cessation of treatment and dosing with control diet for six weeks.
Although the effects observed at this dose level fall within the concentration range for a STOT-RE2 classification, these findings were an adaptive response of the liver and reversible after cessation of treatments and thus considered non-adverse. It is the applicant's opinion that no STOT-RE classification is warranted.

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ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip

Dossier Submitter's Response

Centrilobular hepatocyte enlargement was observed in males at 400 ppm (26.2/29.3 mg/kg bw/day) along with increases in the absolute and relative weights of liver (both sexes) and significant and dose-dependent increases in the plasma cholesterol level in both sexes. These effects show a pattern of liver damage even if they were reversible after recovery period on week 19. It has to be noted that liver hypertrophy starts in the centrilobular hepatocytes, spreading to the intermediate zone as it progresses, and finally observed as diffuse hypertrophy all around the lobule of the liver. Consequently, the Spanish CA considers the liver effects observed in the 90-day rat repeated dose toxicity study as indicative of adversity adverse. However, even if this effect is regarded adverse for liver, the weight of the evidence based on the whole available information on all studies in several species indicate that clofentecine does not cause a pattern of liver toxicity at dose levels below guidance values sufficient for STOT RE classification.

RAC's response

RAC agrees with the proposal for no classification for this endpoint.

OTHER HAZARDS AND ENDPOINTS – Aspiration Hazard

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	13
Comment received				
No comment ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public attachment to ADAMA comments on clofentezine CLH report.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip				
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
24.09.2019	France		MemberState	14
Comment received				
FR: FR agrees with the proposal of classification for environmental hazards and with the proposed chronic M factor value.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Thanks for your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
18.09.2019	Netherlands		MemberState	15
Comment received				
The NL would like to note with regard to degradation that: - Clofentezine is not readily biodegradable as ultimate degradation mounted to 12% after 28 days in a CO2 Evolution test (OECD TG 301B). In a surface water simulation study (OECD TG 309) mineralization was also slow, reaching 11% after 30 days. Primary degradation, however, was rapid with a DT50 of 5.6-7.2 days. Three transformation products were identified AE C593600, 2-CBA and 2-CBZ. For these products only acute aquatic toxicity data were available showing that they are considerably less acutely toxic than the parent substance. Nevertheless, as chronic data are not available, it cannot be ruled out that they are not chronically classifiable. Therefore, agreed to consider clofentezine as not rapidly degradable, despite the rapid primary degradation. The NL CA would like to note with regard to aquatic toxicity that: - Clofentezine is poorly water soluble with experimentally determined water solubility values of 0.0025 and 0.034 mg/L. The higher value was more recently (2010) determined by the generated column elution method (pH5; 20°C) and was assessed as reliable in the RAR. Nevertheless, aquatic studies reporting measured test concentrations that exceed the lower value of 0.0025 mg/L, but not the higher value of 0.034 mg/L, are considered unreliable by the Dossier Submitter. Still the results from the respective studies (e.g.				

96h-LC50 of >0.0146 mg/L for Oncorhyncus mykiss; 48h-EC50 of > 0.001123 mg/L for Daphnia magna) are used as supplementary information. The Dossier Submitter is requested to clarify why these studies are considered as unreliable, or if they are in fact considered as reliable with restrictions (Klimisch score of 2) which would allow their usage as supplementary data. Unreliable studies (Klimisch score of 3) cannot be used for classification purposes, and subsequently there would be data gaps that would need to be addressed.
- Table 49 (summary of acute toxicity data) appears to contain erroneous acute effect concentrations for daphnids.
o Barrett and Arnold (1988) reported a mean measured 48h-EC50 of >0.001123 mg/L. Please clarify where the EC50 of >0.00084 mg/L is based upon.
o The RAR and the CLH dossier report for Lines (1981) a 48h-EC50 of >0.00004 µg/L. However, the RAR reports for the respective study (B.9.2.4.1/02) measured concentrations of 0.08 ppm at 0 hours, 0.01 ppm at 24 hours and 0.08 ppm at 48 hours, and a mean measured concentration of 0.04 ppm, which corresponds to 40 µg/L. Please verify what the correct 48h-EC50 should be.
- The only algal study with clofentezine reports an EC50 of >0.32 mg/L (Oldersma, et al 1983) and no EC10/NOEC. The reported EC50 is based on yield, is expressed as nominal test concentrations, and exceeds water solubility by a factor of ~10. From the RAR summary it appears that actual concentrations were determined, and that they were less than 10% at test end. The mean measured concentrations are not reported though. Furthermore, growth rate was not calculated, and yield (after 94 hours) suggests a dose-response relationship with the control having a yield of 37.96 x 104 cells/mL and the highest treatment 30.42 x 10 4 cells/mL, respectively. Please reflect on these findings and the validity of the algal study. If the original study is available, could you derive a NOEC based on growth rate and express it as a mean measured concentration? If not please address the data gap for acute and chronic classification.
- The RAR and CLH dossier report for the mysid key study (Aufderhide, 2009) a 28d-NOEC of 0.0033 mg/L for the mean total number of young per female that is based on comparison to the negative control (medium only), and not the solvent control. This approach is indeed a worst-case approach, as there is no significant effect when comparison is made to the solvent control. Furthermore, not all treatments exceeding the NOEC, i.e. 0.0134 mg/L, are significantly differing from the negative control (see RAR Vol 3CA-B-9; RMS Comments: B.9.2.5.2/01-02). The RAR justifies this decision as an apparent dose-response cannot undoubtedly be ruled out. This conservative approach can be agreed upon.

Overall, as the conservative NOEC from the key mysid study is considered acceptable, and as other aquatic toxicity studies showed no effects up to water solubility, it seems unlikely to the NL CA that the classification (Chronic Aquatic 1, M=10) will be altered. Nevertheless, the Dossier submitter is requested to consider the above noted issues, and if needed update the proposal.

Dossier Submitter's Response

We aknowledge your comment regarding degradation.
With regard aquatic toxicity:
1.The studies were not considered acceptable for classification purposes as no effects were reported at the highest concentration possible tested, which was the solubility accepted at the time, and for this reason they were only considered as supplementary information. Furthermore, they were not evaluated for Klimsch score.
2.Comments on Table 49
a.Barrett and Arnold (1988) Study:

There is an erratum in Table 49. The EC50 value is 0.00084 mg/L based on the concentration of clofentezine at the end of the test. Based on the mean measured concentration the EC50 is 0.001123 mg/L.
b.The correct EC50 value based on the mean measured concentration is 40 µg/L.
3.Oldersma et.al:
The substance was not found to impair the growth of the alga at nominal concentrations up to its solubility limit in water. By comparison of the growth curves of algal suspensions exposed to the test substances with those of algal control, the no-observed –effect concentration (NOEC) was reported to be 0.032 mg/l, based on growth yield. The study was conducted according to Dutch draf Standard method NEN 6506 and important deviations with OECD 201 were identified. In addition, the actual concentrations measured at the end of the test were less than 10% of nominal.
The information from this study was considered supplementary, since no endpoints based on growth rate were proposed.
4.Auferheide, 2009.
The original study reports a NOEC of 0.0033 mg/L. While recognizing the limitations of the test, as the significant effect was only found for the comparison with the negative control (and not with the solvent control) the NOEC of 0.0033 mg/l was maintained as a conservative approach.
Nevertheless, the applicant submitted additional statistical analysis of the data demonstrating that the water control is not appropriate for statistical comparison and the solvent control is the most suitable to derive a reliable endpoint. The resulting NOEC using the solvent control is 26.9 µg/L. (see response to comment number 18).

RAC's response

1. RAC aknowledges comment on biodegradation
2. Aquatic toxicity
Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) was studied in a flow through system with radiolabelled clofentazine. An EC_{50} > 0.0146 mg/L was found. In light of new data for substance solubiity this concentration is acceptable. However, in this study: only one concentration is tested, substance was firstly absorbed to pumice which was then used, via a saturation column, to supply a constant level of dissolved clofentazine. Clofentazine degrade very fast at pH > 7 to non toxic degradation products – scintillation measurements are not selective to parent compound. The study is valid and might be used as supplementary information. Regarding acute toxicity study to Daphnia Magna – single concentration is tested in a static test - the amount of clofentezine at the start of the test was 1.45 µg/L, but 0.84 µg/L was recorded after 48h; mean calculated concentration 0.001123. The applicant stated that EC_{50} value could not be reached at the tested concentration. The study is valid, but might be used as a supplementary information.
Not enough imformation for Klimisch score assessment.
3. Comments on Table 49
The amount of clofentezine at the start of the test was 1.45 µg/L, but 0.84 µg/L was recorded after 48h; mean calculated concentration 0.001123, EC_{50} > 0.001123
Mean calculated EC_{50} > 0.04 ppm (however measured concentrations were 0.08 ppm at 0 hours, 0.01 ppm at 24 hours and 0.08 ppm at 48 hours (this value might be wrong at the end of the test).
4. Oldersma *et al.*:
RAC has no access to the original GLP study, performed according to the Dutch draft Standard method NEN 6506. RAC is aware that the publication provided in the RAR summary lacked relevant data which RAC cannot verify:

- Effect of clofentezine on algal growth showed some dose-response relationship, but statistical analysis is not presented, standard deviations are not given.

- Measured, actual concentrations are less than 10% of nominal at the end of the test (no data presented).
- No end points for growth rate are available

The applicant concluded that clofentezine in concentrations up to its solubility limit in water did not impair the growth of the alga *Scenedesmus pannonicus but i*n concentrations exceeding that limit it had a slight effect on growth yield. The 120-hour EC_{50} accepted was > 0.32 mg/L and NOEC 0.032 mg/L.
RAC is of opinion that it would be possible to accept this study as supplementary information.
5. Auferheide *et al.*
The applicant submitted new, reliable statistical analysis of values obtained for mean total young per F0-female and clearly demonstrated that NOEC 0.0296 mg/L is a valid endpoint taking into account solvent control. RAC accepts statistical calculations as realiable and agrees that the solvent control should be used instead of negative control.

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2019	United Kingdom		MemberState	16
Comment received				

Clofentezine (ISO); 3,6-bis(o-chlorophenyl)-1,2,4,5-tetrazine (EC: 277-728-2; CAS: 74115-24-5)
Water solubility:
The CLH report includes 2 water solubility values:
1. van Meter (2010) GLP endpoint of 34.2 ug/l at 20oC
2. Smith and Kelly (1985) non-GLP endpoint of 2.52 µg/l at pH5 and < 2µg/l at pH 7 and 9, temperature not quoted.

There is a factor of ~10 difference between these endpoints. Please can you confirm if either value is considered more reliable and a key endpoint. This is important to aid interpretation of the environmental data.

Bioaccumulation:
A fish bioaccumulation study is available which was considered valid in the 2005 DAR. In the RAR 2007 update the reliability of the study was questioned with the principle limitation relating to dissolved test item concentrations noting clofentezine is likely to undergo rapidly hydrolysis and was measured in aqueous media above the water solubility of 0.00252 mg/l. However, the 2010 van Meter water solubility study and the 2016 Göcer hydrolysis study do not support this position.

The CLH report states that the mean measured water concentration was 0.033 mg/l although further details are not presented. The value is similar to the van Meter (2010) water solubility measurement indicating the test system may not have been conducted with aqueous concentrations above the test item solubility. It would be useful to present further measured concentration details to consider this point.

At present we do not think it is possible to consider the study invalid for the purpose of hazard classification. In addition, the data, while not lipid normalised or growth corrected, appear to indicate the hazard classification bioaccumulation criteria is not met.

Chronic toxicity to Americamysis bahia (2009 and 2019 amendment):
Statistically significant effects were only observed when treatment data were compared to the procedural water control using an ANOVA test.

Statistically significant effects were not observed when exposure treatment data were compared to the solvent control using the Williams trend test although the level of p significance is not presented in the CLH report.

No statistical difference between the solvent and procedural controls was observed in the study report although method details of this comparison are not presented. We note that of the 3 procedural control replicates, 1 appears to include a higher number of young although it is unclear if this replicate is an outlier. The higher SD associated with the mean young per female for the procedural control also indicates more variability in the procedural control compared to the solvent control.

We wonder if comparing exposure treatment data with pooled controls (i.e. solvent and procedural controls) would be useful to clarify if there was a statistically significant effect. This should consider if procedural control replicate C is an outlier.

At present we do not consider the data presented in the CLH report supports Aquatic Chronic 1.

Dossier Submitter's Response

1.-The solubility value of 0.00252 mg/l determined by Smith, S, Kelly was the value accepted in CONCLUSION ON PESTICIDE Peer review of the pesticide risk assessment of the active substance clofentezine Issue on 4 June 2009. (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2009.269). However, this solubility study is not accepted anymore, so a solubility of 34.2µg/l must be taken into account, and be used as end-point.
2.-Bioaccumulation
Taking into account the solubility of 0.34 mg/L (Van Meter), study would have not been conducted with aqueous concentrations above the solubility limit. However, additional data are still needed to prove that parent compound concentration in the test chamber is maintained during the uptake phase. Therefore, the Spanish CA cannot consider the calculated mean bioconcentration factor of 248 determined by Hill et al (1987) as reliable for classification purposes.
However, a new OECD 305 (2012) study of bioconcentration factor submitted by the applicant has been evaluated. The study concludes a lipid-normalised growth-corrected kinetic BCF value of 276 L/kg, indicating that clofentezine does not bioaccumulate in fish. The Spanish CA considers the study acceptable.
3.-Chronic toxicity to Americamysis bahia
The original study reports a NOEC of 0.0033 mg/L. While recognizing the limitations of the test, as the significant effect was only found for the comparison with the negative control (and not with the solvent control) the NOEC of 0.0033 mg/l was maintained as a conservative approach.
Nevertheless, the applicant submitted additional statistical analysis of the data demonstrating that the water control is not appropriate for statistical comparison and the solvent control is the most suitable to derive a reliable endpoint. The resulting NOEC using the solvent control is 26.9 µg/L. (see response to comment number 18).

RAC's response

The solubility of clofentezine at 20 ºC is 0.034 mg/L – this value is valid and should be used.
Bioaccumulation
The applicant presented a new study for clofentezine bioaccumulation in fish. RAC accepts the calculated lipid-normalised growth-corrected kinetic BCF of 276 L/kg as valid and concludes that clofentezine is not bioaccumulative in fish.

NOEC *Americamysis bahia*
New statistical analysis of data for chronic toxicity to *Americamysis bahia* showed insignificant effect when solvent control is taken into account. RAC notes that a solvent control should be used in accordance with generally accepted rules for the evaluation of chronic toxicity tests. Therefore, NOEC of 0.0269 mg/L is a valid endpoint for total young per F0-female. Clofentezine should be classified as Aquatic Chronic Category 1, M-factor 1.

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	Belgium		MemberState	17

Comment received

Based on the available data in the CLH dossier, we support the proposal of environmental classification : Aquatic chronic 1, H410 and M chronic=10.

Some editorial or/and minor comments :
It is not clear for
- the acute Daphnia study (Lines D, 1981) which cited EC50 is the correct one. In table 49 an EC50>0.00004µg/L is given, while in the description of the study an EC50>0.08mg/L is mentioned.
- The algae study with metabolites: in table 50 it is mentioned that the Mead and Mulee study (2001) was performed with 2-CBA, while in the description the metabolite 2-chlorobenzonitrile is mentioned

Dossier Submitter's Response

In relation to Daphnia study, please refer to response to comment 15.
We acknowledge the algae study with metabolites comments. The study was performed with 2-CBN instead of 2-CBA.

RAC's response

The valid value for acute Dapnia study is 0.04 mg/L.
The alga study was performed with chlorobenzonitrile.

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	18

Comment received

The applicant has 3 comments with regard this section:
1. CLH REPORT POINT 11.4:
The CLH report states in section 11.4.2 (page 169) in the evaluation of determination of the accumulation and elimination of [14C]-Clofentezine in Bluegill sunfish (Lepomis macrochirus); Clofentezine: Bioconcentration of clofentezine in bluegill sunfish (B.2.2/04-05; B.2.2/04-05. In DAR 2005):
"In the absence of a reliable bioaccumulation study, the information of the octanol/water partition coefficient should be taken into account to evaluate the substance´s bioaccumulation potential. Already at the DAR and addenda in 2005, as well as at the EFSA Conclusion in 2009, the log Kow value of 4.09 was accepted. This log Kow can be considered to reflect the bioaccumulation potential of clofentezine.."
In due course of the EU evaluation for renewal of clofentezine registration (EFSA request 72 for additional information), the applicant submitted a new study for bioconcentration of clofentezine in fish (XXXXXXXX 2019, ADAMA reference number 0001015500). An OECD summary of this study is included in the attachment (see file ADAMA_002 in the

attachment).
This new study compliant with guideline OECD 305 (2012) concludes lipid-normalised growth-corrected kinetic BCF values of 276 L/kg for spiked aqueous solution and RMS evaluated the study acceptable (revised dRAR of July 2019).

2. CLH REPORT POINT 11.6.2:
The CLH report states in section 11.6.2 (page 184) in the findings of the life-cycle toxicity test of the saltwater mysid, Americamysis bahia, conducted under flow-through conditions (Aufderheide, J. 2009; 2016):
"The number of young per female mysid was the only biological parameter that resulted in a statistically significant difference when compared to the negative control data at the concentrations of 6.65 and 26.9 µg clofentezine/L (mean measured concentrations). Therefore, the NOEC value determined for mean number of total young produced per female was 3.30 µg clofentezine/L.
The applicant submitted an additional statistical analysis (the Williams' trend test) using the vehicle control data instead of dilution water control (in accordance with the OECD Number 54 Guidance document). Based on the results from this new test, no statistically significant reduction in the reproductive data for any of the treatment levels tested were determined. Subsequently, the NOEC value for mean total number of young per F0-female mysid resulted in 26.9 µg/L.
However, an apparent dose-response cannot be undoubtedly ruled out, and in this case, the RMS proposes to maintain the NOEC=3.30 µg/L as a conservative approach. In the opinion of RMS, this endpoint is clearly conservative as no statistical significant effects were observed at any treatment level when they are compared to vehicle control which is a most realistic approach than the comparation with water control.
The endpoint accepted was 28d- NOEC Mean total young per f0-female = 0.0033 mg a.s./L*, which is considered a conservative approach."
In due course of the EU evaluation for renewal of clofentezine registration (EFSA requests 85 and 86 for additional information), the applicant submitted additional statistical evaluations and data analyses demonstrating that the water control is not appropriate for statistical comparison and the solvent control is the most suitable to derive a reliable endpoint with a NOEC of 26.9 µg/L. The data review and statistical evaluations are included in the attachment (see files ADAMA_003 and ADAMA_004 in the attachment).
The RMS agrees with the applicant's response and is of the opinion that "a NOEC of 26.9 µg/L is the most appropriate chronic endpoint for A. bahia" (revised dRAR of July 2019).
Therefore, the endpoint accepted is 28 d-NOEC mean total young per f0-female = 0.0269 mg a.s./L.
3. CLH REPORT POINTS 11.7 and 11.8:
Based on the additional information submitted in the EFSA data call-in (May 2019) as commented above, the content of Table 51 in section 11.7 of CLH report, page 189, shall be changed with respect to the following:
- clofentezine data for long-term toxicity: "Invertebrates' NOEC = 0.0269 mg a.s./L". (This change applies as well in chapter 11.8, page 192)
- CLP classification criteria for bioaccumulation factor: "lipid-normalised growth-corrected kinetic BCF values of 276 L/kg", "Experimental BCF value considered valid", "No bioaccumulative potential"
The conclusions on classification and labelling for environmental hazards in section 11.8 of the CLH report, page 192, may be amended for the M-factor concluding in M = 1 (replacing the currently proposed M =10).

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public attachment to ADA1MA comments on clofentezine CLH report.zip

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip

Dossier Submitter's Response

1.- The new OECD 305 (2012) study of bioconcentration factor submitted by the applicant has been evaluated. The study concludes a lipid-normalised growth-corrected kinetic BCF value of 276 L/kg, indicating that clofentezine does not bioaccumulate in fish. The Spanish CA considers the study acceptable.
2.- The new statistical analysis of the study with Americamysis bahia submitted has been revised, demonstrating that the solvent control is the most appropriate for statistical comparison. Using the solvent control, the resulting NOEC is 26.9.
The Spanish CA agrees with the conclusions of the the applicant. Therefore, this NOEC of 26.9 µg/L should be used for classification purposes.
3.-Taking into account the additional informations submitted, table 5 should be:

Endpoint	CLP classification criteria	Clofentezine data	Conclusion
Water solubility	-	0.034 mg/L	Poorly soluble
Rapid degradability	Demostrated rapid/not rapid degradation	Not readily biodegradable and not rapidly degradable	Not rapidly degradable
Short-term toxicity	LC_{50}/EC_{50} value	No adequate data for fish, invertebrates nor algae. Only considered as additional information.	No acute toxicity recorded up to the water solubility
Long-term toxicity	NOEC value	Invertebrates' NOEC=0.00269 mg/L. No adequate data for fish nor for algae.	One chronic toxicity data available
Bioaccumulation potential	BCF ≥ 500, or if absent, log Kow ≥ 4	lipid-normalised growth-corrected kinetic BCF values of 276 L/kg	No Bioaccumulative potential

Therefore, the final classification of Clofentezine should be Aquatic Chronic category 1; M=1 ; H410 – Very toxic to aquatic life with long lasting effects.

RAC's response

The applicant present a new valid study for bioaccumulation of clofenezine - calculated lipid normalized BCF is 276 L/kg – clofentenzine is not bioaccumulative in fish.
Reliable statistical analysis (the Williams' trend test) of data for chronic toxicity of clofentenzite to *Americamysis bahia* shows NOEC of 0.0296 mg/L (solvent control should be taken into account).
RAC conclude that chlofentenzine should be classified as Acute Chronic Category 1, M-factor 1.

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2019	France		MemberState	19
Comment received				
FR: p14: 8.15.3 Corrosive to metals: the conclusion should be replaced by "Clofentezine is not corrosive to metals". Overall, FR agrees with the proposal of classification for physical hazards.				
Dossier Submitter's Response				
ES: Agree, the sentence is an *erratum* and as stated, it should read "Clofentezine is not corrosive to metals".				
RAC's response				
RAC agrees, "Clofentezine is not corrosive to metals".				

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Germany		MemberState	20
Comment received				
8.1 Explosives and 8.7 Self-reactive substances: The evaluation that Clofentezine is not a self-reactive or explosive substance and does not require classification was not based on the CLP criteria and was not adequately justified. One negative study performed in accordance with EEC A.14 was provided. However, the conclusion that a negative result from the EEC A.14 also automatically means that it does not have to be classified as explosive under CLP is not correct. The test procedures for the classification of explosives are described in detail in the Part I of the UN RTDG, Manual of Tests and Criteria which are not comparable to the classification procedure according to test method A.14 as described in Regulation (EC) No 440/2008 (former Annex V to DSD). In a first step screening procedure should be used for substances which are suspected of having explosive or self-reactive properties. Please, cf. screening procedures in Appendix 6 of the UN-MTC (Reference: UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria, Sixth Revised Edition, New York and Geneva: United Nations, 2015, ISBN 978-92-1-139155-8, ST/SG/AC.10/11/Rev.6.) The classification procedures for explosives need not be applied in accordance with the criteria given in section 2.1.4.3 of Annex I to Regulation (EC) No 1272/2008 if: (a) There are no chemical groups associated with explosive properties present in the molecule. Examples of groups which may indicate explosive properties are given in Table A6.1 in Appendix 6 of the UN RTDG, Manual of Tests and Criteria; or (b) The substance contains chemical groups associated with explosive properties which include oxygen and the calculated oxygen balance is less than - 200; The oxygen balance is calculated for the chemical reaction: C x H y O z + [x+ (y/4)-(z/2)] O 2 → x CO 2 + (y/2) H 2 O Using the formula: Oxygen balance = -1 600 [2x + (y/2)-z]/molecular weight; (c) When the organic substance or a homogenous mixture of organic substances contains chemical groups associated with explosive properties but the exothermic decomposition energy is less than 500 J/g and the onset of exothermic decomposition is below 500 °C. The exothermic decomposition energy can be determined using a suitable calorimetric technique				

The classification procedures for self-reactive substances and mixtures need not be applied in accordance with the criteria given in section 2.8.4.2 of Annex I to Regulation (EC) No 1272/2008 if:
(a) There are no chemical groups present in the molecule associated with explosive or self-reactive properties. Examples of such groups are given in Tables A6.1 and A6.2 in Appendix 6 of the UN RTDG, Manual of Tests and Criteria (Fifth Revised Edition, 2009); or
(b) For a single organic substance or a homogeneous mixture of organic substances, the estimated SADT for a 50 kg package is greater than 75 °C or the exothermic decomposition energy is less than 300 J/g. The onset temperature and decomposition energy can be estimated using a suitable calorimetric technique (see Part II, sub-section 20.3.3.3 of the UN RTDG, Manual of Tests and Criteria).

Clofentezine is a nitrogen-rich substance which is thermally unstable having a melting range 180 – 195 °C followed by exothermic decomposition in the temperature range 190-250 °C.

Thermally unstable substances or mixtures that are not classified as explosives should be considered for classification as self-reactive substances and mixtures.

Based on a literature source on nitrogen-rich substances (see attached Publication by Löbbecke, 1999), the exothermic decomposition energy of diaryl-substituted tetrazines is determined at about 300 J/g.
So far, neither the exothermic decomposition energy nor the SADT have been determined by Clofentezine.
As a minimum requirement a DSC measurement should be provided for justifying that the classification procedures for explosives or self-reactive substances does not need to be performed in the case that the exothermic decomposition energy is less than 300 J/g.

Recommendation on testing: Study to determine the exothermic decomposition energy and if this is higher than 300 J/g (but less than 500 J/g) also the SADT including the classification procedure for self-reactive substances should be provided.

Please, make the following changes to the CLH Report:
Table 6:
Explosives: Reason for no classification: data lacking
Self-reactive substances: Reason for no classification: data lacking
8.1.3 Conclusion on classification and labelling for explosive properties: Data lacking.

8.7.3. Conclusion on classification and labelling for self-reactive substances: Data lacking.

8.10 Self-heating substances:

The criteria for self-heating substances and mixtures are found in Annex I, Section 2.11 of CLP.
EU test method A.16 as described in Regulation (EC) No 440/2008 checks for self-heating properties. However, the method used is generally inappropriate for a sound assessment, and the findings do not lead to a classification and for Clofentezine the evaluation according to Method A. 16 was not carried out correctly.

The measurement of the temperature up to 423 °C is not possible if the substance has a melting range 180 – 195 °C followed by decomposition in the temperature range 190-250 °C.

The principle of the Method A.16 is that the temperatures of the oven and sample are continuously recorded while the temperature of the oven is increased to 400 °C, or to the melting point if lower, at a rate of 0,5 °C/min. The cube (made of stainless steel wire mesh) is filled with the substance to be tested and thermocouple is placed at the centre of the cube.
However, temperature measurement in the melt is no longer possible because the thermocouple measures the temperature of the empty wire basket.
If self-heating behaviour cannot be ruled out by a screening test, further testing becomes necessary using UN Test N.4 in Part III, Sub-section 33.3.1.6 of the UN-MTC.
For safety reasons, it is advisable to test for explosive and self-reactive properties for Clofentezine before performing this test.
Before starting UN Test N.4, the decomposition behaviour of Clofentezine should be known. In general, it is sufficient to perform a screening with Differential Scanning Calorimetry. Special care with respect to the interpretation of the test data is necessary when exothermic decomposition may occur at the test temperatures. In such cases, a test under an inert atmosphere (i.e. nitrogen) should be run to determine the temperature rise due to decomposition. Careful flushing with the chosen inert gas is essential in such cases since otherwise much air may be retained between the crystals of the sample in the container.

Recommendation on testing: Study to determine the exothermic decomposition energy is needed to check for self-reactive properties before performing the UN Test N.4.
Please, make the following changes to the CLH Report:
Table 6:
Self-heating substances: Reason for no classification: data lacking
8.10 Self-heating substances
Table 10, column 2 "Results": replace "No self-ignition temperature up to 423 °C." with "No self-ignition temperature up to the melting range (180-195 °C)."

8.10.3 Conclusion on classification and labelling for self-heating substances: Data lacking.

Dossier Submitter's Response

ES: We agree with the comments.

Re. 8.1 Explosives.
Despite the lack of concern about the explosive properties of clofentezine based on the outcome of the test study method EC A.14 and handling experience, the criteria for non classification stated in the Regulation EC 1272/2008 are not fulfilled. Furthermore, Clofentezine is a tetrazine derivative and it contains contiguous nitrogen atoms, hence the criteria for exclusion according to section 2.1.4.3 of Annex I of Regulation EC 1272/2008 are not met. Therefore, we agree with the "data lacking" statement.

Re. 8.7 Self-reactive
We agree there is a data gap to evaluate this property. A self-reactive substance corresponds to a thermally unstable solid liable to undergo a strongly exothermic decomposition even without participation of oxygen (air). Clofentezine is thermally unstable having a melting point range 180-195ºC followed by exothermic decomposition within the range 190-250ºC. No suitable data are available to evaluate this property and therefore, we agree with the "data lacking" statement.

Re. 8.10 Self-heating substances
We agree there is a data gap to evaluate this property. The test method EC A.16 is not deemed appropriate to evaluate self heating of solids towards CLP classification. No data

are available to evaluate this property and therefore, we agree with the "data lacking" statement.

RAC's response

RAC agrees in general with MS comments for appropriate data lacking, however, notes some important points for chlofentenzine, regarding explosive, self-reactive and self-heating properties.

8.1 Explosives:
1. Clofentezine does not contain aliphatic azo groups (-R-N=N-R-) shown in Table A6.1 of UN RTGD, indicating explosive properties.
2. Clofentezine is not strictly speaking nitrogen rich compound – nitrogen content 18%.
3. In the paper, Löbbecke, 1999, none of the studied diaryl-substituted tetrazines showed exothermic decomposition energy above 300 J/g. Furthermore, according to the mechanism of decomposition of diaryl-substituted tetrazines in this study, explosive decomposition should not be expected. Almost the same should be valid for clofentezine
4. In the Bretherick's Handbooks no examples for explosive diaryl-substituted tetrazines are given.
5. A negative EC A.14 study is available and might be accepted as supportive evidence.
6. The substance has beens on the market for more than 15 years without incidents.
RAC is of opinion that based on above mention facts it might be concluded that clofentenzine deserves "no classification" for explosive properties.
Thermally unstable substances or mixtures that are not classified as explosives should be considered for classification as self-reactive substances and mixtures.
RAC noted the following:
1. Clofentezine does not contain aliphatic azo groups shown in Table A6.1 of UN RTGD.
2. Clofentezine does not contain chemical groups shown in Table A6.3 UN RTGD indicating self-reactive properties.
RAC acceptes the MS recommendation on testing for accurate determination of the exothermic decomposition energy and SADT. RAC cannot recommend a classification due to lack of data.

Self-heating substances:
RAC agrees that the EU test method A.16 is in general inappropriate for substances with low melting points (Guidance on the application of CLP criteria, 2017).
The conclusion from the test method A.16 should be: No self-ignition temperature up to the melting range (180-195 °C). RAC cannot recommend a classification due to lack of data.

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	France	ADAMA Agriculture BV	Company-Manufacturer	21

Comment received

No comment

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public attachment to ADAMA comments on clofentezine CLH report.zip
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential attachment to ADAMA comments on clofentezine CLH report.zip

Dossier Submitter's Response

Noted.

RAC's response
Noted.

PUBLIC ATTACHMENTS
1. Public attachment to ADAMA comments on clofentezine CLH report.zip [Please refer to comment No. 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 18, 21]

CONFIDENTIAL ATTACHMENTS
1. Confidential attachment to ADAMA comments on clofentezine CLH report.zip [Please refer to comment No. 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 18, 21]