Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

quinoclamine (ISO); 2-amino-3-chloro-1,4-naphthoquinone

EC Number: 220-529-2
CAS Number: 2797-51-5

CLH-O-0000006853-67-01/F

Adopted

17 September 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON QUINOCLAMINE (ISO); 2-AMINO-3-CHLORO-1,4-NAPHTHOQUINONE

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.

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Substance name: quinoclamine (ISO); 2-amino-3-chloro-1,4-naphthoquinone
EC number: 220-529-2
CAS number: 2797-51-5
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
02.08.2019	Belgium		MemberState	1
Comment received				
We want to thank SE CA for submission of the CLH proposal for Quinoclamine				
Dossier Submitter's Response				
Thank you!				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2019	Germany		MemberState	2
Comment received				
The proposed classification of quinoclamine as Carc. 2, H351 is based on benign transitional cell papillomas in urinary bladder and benign phaeochromocytoma in adrenals in SD rats of both sexes. In addition, malignant lymphoma was noted in female CD-1 mice. The incidence of malignant lymphoma in mice was not that convincing. The German CA would appreciate if the dossier submitter could deliver information on the number of female mice examined for comparison with the historical control data. Furthermore, in the highest dose group (300 ppm) the body weight gain in females was reduced by 30 %. Therefore the MTD is exceeded. Regarding the benign tumours at multiple sites in SD rats, in the highest dose group (676 ppm) the MTD is exceeded for females (reduced body weight gain of 27 %). It has also to be noted that there is a high spontaneous tumour incidence for adrenal				

pheochromocytoma in male SD rats. From our point of view the increased incidences of benign tumours at multiple sites in SD rats are considered as borderline evidence between category 2 and no classification.
Dossier Submitter's Response
The number of female mice examined for histopathology was 50 animals/group. The indicidence of malignant lymphoma noted in the mouse at the highest dose of 300 ppm (12/50=24%) was just slightly outside the historical control data (22%) for the study report but showed a statistically significant trend. It is proposed that the positive trend supports the proposed classification as Carc cat 2. The benign tumours in the rat (Crl:CD(SD)BR) were noted at the highest dose level (676 ppm). At this dose level body weight gain was adversely reduced (27%) in females only. No effects on body weight were noted in the males. Furthermore, no mortality or adverse clinical signs were noted at this dose level, thus the MTD seems not been exceeded. It is proposed that the benign tumours in the rat still is considered as concern for classification as Carc cat 2.
RAC's response
RAC agrees with the MSCA that the mouse lymphoma incidence in light of the normal variable and high background level for this tumour may be considered unconvincing. Similarly the incidence of rat pheochromocytoma is also not that convincing. That leaves just one tumour type with a clear substance effect and with no evidence of progression to malignancy. This is a borderline case but considering the very strong hyperplastic response in the urinary bladder without any mechanistic information to suggest otherwise, there are very clear treatment effects leading to benign tumours that must be considered as potentially relevant for human health. Carc 2. is proposed based on one tumour type, transitional cell papilloma, in the rat urinary bladder.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2019	Germany		MemberState	3
Comment received				
The German CA agrees with the dossier submitter that no conclusion on classification and labelling for genotoxicity/germ cell mutagenicity could be drawn because the data were inconclusive.				
Dossier Submitter's Response				
Noted				
RAC's response				
RAC agrees with the Member State comment.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
30.07.2019	Netherlands		MemberState	4
Comment received				
The NL MSCA agrees with the proposed 'no classification' for adverse effects on fertility and for adverse effects on or via lactation. With respect to adverse effects on development, multiple effects were observed. These included a.o. aortic arch malformations, skeletal abnormalities and effects on kidney.				

An increased incidence (though being low) of aortic arch malformations was noted. This was observed in multiple studies and in both rat and rabbit, although it could not be reproduced in all studies. At some dose levels aortic arch malformations occurred in the absence of (marked) maternal toxicity. We agree that also the skeletal abnormalities and kidney effects cannot be fully explained by (marked) maternal toxicity. Overall, these effects are considered severe and relevant for classification. However given the uncertainties, the NL MSCA agrees with the proposed classification as Repr. 2 (H361d). It is noted that page 175 (section 2.6.6.2.2) and page 179 (section 2.6.6.4) of the CLH-report incorrectly present the hazard-statement H361d as "Suspected of damaging fertility or the unborn child".
Dossier Submitter's Response
Noted
RAC's response
RAC agrees with the comment. RAC supports the proposal for Repr. 2 (H361d).

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2019	Germany		MemberState	5
Comment received				
The submitted study on acute inhalation toxicity was considered not acceptable by the Dossier Submitter due to low amounts of respirable particles, taking into consideration that the mode of exposure was whole-body and not nose-only which is recommended in the OECD TG 403. However, the study was already performed in 1986. Whole-body exposure was acceptable in this time according to OECD TG 403. According to the study report, 0.79 mg/L was the highest attainable concentration in the only available study and only about 40 % by weight of the test substance was 5.5 µm or less. Based on the available results, quinoclamine cannot be allocated to a toxicity category according to the CLP guidance. Therefore, the German CA agrees that no conclusion on classification and labelling for acute inhalation toxicity could be drawn.				
Dossier Submitter's Response				
Noted				
RAC's response				
RAC agrees with the comment from the MSCA.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
16.08.2019	United Kingdom		MemberState	6
Comment received				
Quinoclamine (ISO); 2-amino-3-chloro-1,4-naphthoquinone (EC: 220-529-2; CAS: 2797-51-5). Acute toxicity to fish using the active ingredient: We are unclear why 0 and 90% mortality treatments were used to calculate the acute fish LC50 in the 1991 study as we note a 100% mortality treatment is available. It may also be possible to use current software to statistically determine a LC50 based on the				

observed dose-response.

In addition, the quoted 0.044 mg/L LC50 endpoint is based on measured concentrations at 48 hours only although the study is described as semi-static and measured concentrations are available for 0 and 24 hours.

We wonder if it would be possible to consider 0, 24 and 48 hour renewal and measured concentrations with estimated losses at 96 hours to assess 0-96 hour measured concentrations – potentially as a time-weighted average. A rough calculation indicates this approach is likely to result in an LC50 within the same 0.01-0.1 mg/L classification range.

This information is relevant as it relates an endpoint using the active ingredient in the 0.01 to 0.1 mg/L classification range supporting Acute 1 classification which is proposed currently on the basis of an algal study using the formulation (see comments relating to the endpoint below).

Chronic toxicity to fish using the active ingredient:
We consider the EC10 endpoints should be considered in preference to the 0.00213 mg/L NOEC endpoints - this does not change the classification proposal.

We note that EC10 endpoints are not presented for other response endpoints with the same 0.00213 mg/L NOEC. These should be presented if available as the study is the key Aquatic Chronic 1 classification although we recognise that such endpoints will not change the classification/M-factor.

Acute toxicity to invertebrates using the active ingredient:
A 1991 48h immobilisation study following OECD 202 using the active ingredient and Daphnia magna is available in the DAR but not presented in the CLH. The study met validity criteria although analytical verification was only undertaken at 0h for some treatments (94-98% of nominal). While the lack of analytical support impacts the reliability of the 48-h EC50 2.15 mg/L, we note that the treatments appear to have been correctly dosed and given losses observed in other aquatic media over acute timescales, we consider it is unlikely that mean measured concentrations would result in an EC50 below 1 mg/L. On this basis, we consider invertebrates are not the most acutely sensitive species

Chronic toxicity to C. riparius study using the active ingredient:
Given the significant partitioning from water to the sediment phase over the study period, we do not consider the quoted endpoint is reliable for hazard classification.

Chronic toxicity to invertebrates:
Given we do not consider the C. riparius study provides a reliable endpoint, the chronic classification to invertebrates should consider the surrogate approach using acute information for the active ingredient. We note this will not impact the classification proposal. We note this data gap and highlight that the invalid 1994 chronic toxicity to Daphnia magna study indicates the active substance may exhibit chronic toxicity to invertebrates.

Algal growth inhibition using the active ingredient:
The DAR includes 2 growth inhibitions studies using the active substance: Jahnke, 1994 using S. subspicatus and Barth, 2000 using N. pelliculosa. Both are considered invalid as the controls are not considered to meet current OECD TG 201 validity criteria for ≤35%

CoV for section-by section growth rates for 0-72 hours.

We consider that these studies are relevant to the classification as the endpoints may be lower than the acute fish and invertebrate endpoints for the active substance and the algal endpoint using the formulation. Therefore, we think further analysis of control validity is required.

In the first study (Jahnke, 1994) raw cell data is seemingly not readily available to calculate the criteria endpoint and mean values potentially indicate failure. Aside from this issue, the study is described as valid with a 72-h ErC50 of 0.022 mg/L and 72-h ErC10 of 0.0075 mg/L. It may be that the raw data are now available. If not, it is possible to consider 48h ecotoxicity endpoints as the mean cell data indicates the controls are valid at that point. This approach is considered valid in the test guideline and has previously been applied for hazard classification. On this basis, please can the DS clarify if the control data are now available and if not present 48 hour endpoints? This information is important as it would take precedence over formulation data and in the case of the Acute classification may drive the classification.

For the Barth, 2000 study, full control data are not presented in the DAR so it is unclear whether the quoted 43% CoV is driven by cell counts between 48 and 72 hours which is often the case. The study is also described as valid aside from this issue. Please can the DS consider 0-48 hour controls were valid and if endpoints can be generated for this period?

Algal growth inhibition study with formulation:
The proposed acute classification is on the basis on this study 72-h ErC of 0.029mg a.s./L using Scenedesmus subspicatus. A chronic NOEC/EC10 endpoint is not presented – we consider a NOEC and/or EC10 based on geometric mean measured concentrations could be statistically derived and estimate a NOEC would be in the range 0.001 to 0.1 mg a.s./L. We note this NOEC is in the same concentration range as the fish chronic NOEC which is used for the proposed classification.

The nominal concentration of quinoclamine reported for the 0.04 mg/L nominal concentration of Mogeton 50% WG appears to be based on a different percentage content of the active substance to the other treatment levels. Please could the DS clarify the content of quinoclamine in the Mogeton formulation used?

Lemna study with active substance:
In the DAR (2007), the Lemna study by Kleiner (2000) has an ErC50 of 0.09 mg a.s./L and ErC10 of 0.03 mg a.s./L, whereas the EbC50 is 0.11 mg a.s./L and the EbC10 is 0.05 mg a.s./L. The same ErC50 of 0.09 mg a.s./L and EbC50 of 0.11 mg a.s./L are reported in the EFSA conclusion (2007). These results are inconsistent with the study results in the CLH with an ErC50 of 0.11 mg a.s./L, ErC10 0.05 mg a.s./L and NOErC 0.04 mg a.s./L. It appears a mistake was made when the studies were copied across to the RAR and CLH. This is important because ErC50 values are preferred over EbC50 values for classification purposes and the ErC50 at 0.09 mg a.s./L is in the same concentration range as the lowest acute endpoints.

Ecotoxicity studies using the formulation studies:
The CLH states there are no indications from the available data that the co-formulants in the product are more toxic or increase the toxicity of Quinoclamine to aquatic organisms. Does this mean that full ecotoxicity data are available for the co-formulants or is there additional information to support the statement? This is relevant as the DS proposes that

the acute classification is based on a study using the formulation.

Dossier Submitter's Response

Acute toxicity to fish using the active ingredient:
Due to the selected doses in the test, there were no mortality rates in between 0 and 90%. Therefore, any statistically determined probit analyses would be of very low power. Using the geomean of concentrations at 48 hours with 0 and 100% mortality as an alternative would result in an LC50 of 0.064 mg/L, ie still in the range 0.01-0.1 mg/L.

Since there was no clear declination pattern, any extrapolation of test concentrations at 72 and 96 hours would be very uncertain. On the other hand, using geomean measured concentrations from the first 48 hours of the test would most likely result in an over-estimation of the overall geomean during the test. Therefore, given the available information, we would maintain that the 48 h measured concentrations are the most appropriate for derivation of the LC50 in this case.

Chronic toxicity to fish using the active ingredient:
EC10 was available only for post-hatch survival 90 dpf and total survival 90 dpf (0.00255 and 0.00240 mg/L, respectively), while for other sensitive parameters (post-hatch survival swim-up phase and length 90 dpf no EC10 values were available. Therefore, we would prefer to maintain the NOEC value as the most appropriate in this case.

Acute toxicity to invertebrates using the active ingredient:
Although we maintain that the lack of analytical support in the study with the active ingredient makes the results uncertain, we agree that invertebrates can be concluded as not being the most acutely sensitive aquatic group of species. This is supported by the available data on the tested formulations (48h LC50 corresponding to ca 1 mg a.s./L).

Chronic toxicity to aquatic invertebrates using the active ingredient:
We note that the quoted endpoint from the C. riparius study, as well as the available chronic data for Daphnia magna can be considered as less appropriate for hazard classification. Therefore, the chronic hazard for aquatic invertebrates is not fully addressed.

Algal growth inhibition using the active ingredient:
Unfortunately, the raw cell data from the study by Jahnke (1994) is still missing. Since cell counts are presented only as mean and standard deviations, it is not possible to calculate CV for the section by section growth rate. For this reason, we maintain that the study does not fulfil the validity criteria, neither at 72h or 48h. Nevertheless, the results may possibly be used as supportive information for the hazard assessment, with a 72h ErC50 of 0,022 mg/L (nominal), which is similar to the corresponding (reliable) study with the formulated product.

For the Barth, 2000 study, a 48h ErC50 of 0.768 mg/L was reported, based on measured concentrations on day 0. However the test levels decreased to ca 60% at the end of the test, and no analytical measurements were made at 48 hours. Further, also at 24 and 48 hours, the control variability seems to be higher than the recommended upper limit of 35%. We would not rely on these data for the hazard assessment.

Algal growth inhibition study with formulation:
The chronic (72h) NOEC for Scenedesmus subspicatus was 0.02 mg formulation/L, corresponding to a nominal concentration of 0.01 mg a.s./L. At this test level, no analytical measurements were made. However, assuming a similar pattern of as for

available data as at the next higher test level (where geomean measured 74% of the nominal value) would result in an estimated 72h NOEC of 0.007 mg a.s./L.

The tested Mogeton formulation is a water dispersable granulate with a nominal content of 50% active ingredient. The discrepancy between the reported nominal test concentrations was probably due to a slight difference between %weight and %volume content of the formulation.

Lemna study with active substance:
We have revisited the original study for confirmation. The correct values are: ErC50 0.11 mg a.s./L and ErC10 0.05 mg a.s./L, EbC50 0.09 mg a.s./L and EbC10 0.03 mg a.s./L. These values are consistent with those presented in the draft RAR (2018) and in the CLH report, while those given in the previous DAR/EFSA conclusion (2007) were incorrect.

Ecotoxicity studies using the formulation studies:
No full ecotoxicity data is available for the co-formulants in the product. The proposal that there are no indications from the available data that the co-formulants in the product are more toxic or increase the toxicity of Quinoclamine was based on results from comparable studies with active ingredient and formulation where available.

RAC's response

Acute toxicity to fish using the active ingredient:
RAC agrees with the DS answer to use 48-h LC50 value for classification,

Chronic toxicity to fish using the active ingredient:
The NOEC of 0.00213 mg/L used by the DS is for post-hatch survival swim-up phase, post-hatch survival 90 dpf, total survival 90 dpf and length 90 dpf. The EC10 values have been calculated for post-hatch survival and total survival. None of these endpoints seem to be more sensitive than the other. Therefore, RAC agrees with the commenting MS that the EC10 should be used for classification.

Acute toxicity to invertebrates using the active ingredient:
RAC agrees with the commenting MS to conclude that invertebrates are not the most acutely sensitive species.

Chronic toxicity to C. riparius study using the active ingredient:
RAC agrees to the commenting MS comment that given the significant partitioning from water to the sediment phase over the study period, we do not consider the quoted endpoint is reliable for hazard classification.

Chronic toxicity to invertebrates:
RAC agrees to the MS comment that surrogate approach using acute information for the active ingredient should be considered for classification.

Algal growth inhibition using the active ingredient:
RAC agrees to the DS to the fact that the Jahnke 1994 and Barth 2000 studies are not valid.

Lemna study with active substance:
RAC welcomes the confirmation of the values presented in the CLH Report: ErC50 0.11 mg a.s./L and ErC10 0.05 mg a.s./L, EbC50 0.09 mg a.s./L and EbC10 0.03 mg a.s./L. These values are consistent with those presented in the draft RAR (2018) and in the CLH report, while those given in the previous DAR/EFSA conclusion (2007) were incorrect.

Ecotoxicity studies using the formulation studies:
RAC notes the DS statement that no full ecotoxicity data is available on the co-formulants in the product and the fact that the proposal to use toxicity data from product tests is based on comparable results from the studies with active ingredient and formulation. RAC, however, disagrees to use product data for the substance classification based on the uncertainties related to possible effect of co-formulants to the toxicity in the product test.

Date	Country	Organisation	Type of Organisation	Comment number
02.08.2019	Belgium		MemberState	7

Comment received

BE CA supports the proposed environmental classification for quinoclamine:
Aquatic Acute 1, H400; M-factor=10 (0.01<EC50≤0.1 mg/L)
Aquatic Chronic 1, H410; M-factor=10 (0.001<NOEC/EC10≤0.01, NRD)

Quinoclamine is a substance that impacts the photosynthesis of algae. Determination of the acute M-factors is based on the result of an algae study though using Mogeton 50% WG, a formulation with quinoclamine. Although not impacting the proposed M-factor, we suggest the conversion of the EC50 to reflect the technical grade of 99%, resulting in a 72hEC50 = 0.0146 mg/L.

Furthermore, a.o. on p. 16 of the CLH report it is mentioned that the active substance contains a relevant impurity : dichlone. Dichlone has an Harmonised classification and labelling: Aquatic Acute 1, H400 and Aquatic Chronic 1, H410. We want to note that when the presence of this substance is ≥0.1%, this needs to be take into account for classification. However no harmonised M-factors are available for this impurity. Dichlone is self-classified with M=10 both for acute and chronic aquatic toxicity.

Dossier Submitter's Response

Noted. We do not fully understand the comment regarding conversion of the EC50 for algae. The ErC50 of 0.029 mg a.s./L reported in the CLH report was already corrected for the content of active ingredient in the formulation.
Regarding the impurity of quinoclamine, from our understanding the small amounts present would not have an impact on the classification of the substance.

RAC's response

It is said in the CLH report that dichlone is present at max. 1.5%. Following CLP 1.1.2.2.2 and 4.1.3.1, a concentration of 1.5% would not be enough to warrant a Aquatic Acute 1 and Aquatic Chronic 1 classification to quinoclamine. In case dichlone would have an M-factor of 10 the limit for Aquatic Acute 1 and Aquatic Chronic 1 classification would be 2.5%.

Date	Country	Organisation	Type of Organisation	Comment number
30.07.2019	Netherlands		MemberState	8

Comment received

In principle, we support the proposal to classify quinoclamine as Aquatic Acute 1 (M-10) and Aquatic Chronic 1 (M=10). However, we do not agree with the choice of key studies used for the classification of the substance.

Acute aquatic hazard
p. 283 the selection of key data for invertebrate and algae

We do not agree with the use of toxicity data for invertebrates (D. magna 48h EC50 = 1.03 mg/L, Table 2.9.2.2-1) and algae (S. subspicatus 72 ErC50 = 0.029 mg/L, Table 2.9.2.3-1) as key data for these trophic levels. In our opinion, the use of the toxicity data based on the formulated product (Mogeton 50% WG) is not appropriate for classification purposes. In general, the classification of a substance is based on test data from the substance itself. In studies conducted with formulated products, it cannot be excluded that effects can at least partially be attributed to other constituents of the formulations. The dossier submitter has not provided a justification as to why the studies conducted with the formulation is adequate to conclude on the active substance.
Reliable short-term aquatic toxicity data on quinoclamine are available for fish (Rainbow trout) with a 96h LC50 value of 0.044 mg a.s./L (measured) and for aquatic plant (L. minor) with a 7d ErC50 value of 0.11 mg a.s./L (geomean measured). There is no reliable data for invertebrates. The most sensitive trophic group is fish and on this basis quinoclamine should be classified as: Acute category 1. The endpoint being in the range of 0.01 mg/L <L/EC50 ≤0.1 mg/L, the acute M-factor is 10.

Chronic aquatic hazard
p. 284 Section 2.9.2.4.2, we agree with the conclusion that quinoclamine is not rapidly degradable and has a low potential to bioaccumulate.

p. 283 the selection of key data for aquatic plants
We do not agree with the use of toxicity data for aquatic macrophyte (M. spicatum 14d EC50, root number = 0.515 mg a.s./L, Table 2.9.2.3-1) as key data for the classification of quinoclamine. From our perspective, the undertaken test study (OECD Test Guideline 238) with test species (M. spicatum) is not suitable for classification purposes. The most commonly used vascular plants for aquatic toxicity test are duckweeds (Lemna gibba and L. minor) and the observational endpoint is based on change in the number of fronds produced (CLP guidance section I.2.3.2).
A reliable aquatic toxicity test is available for aquatic plant (L. minor) with a 7d ErC10 value of 0.05 (geomean measured). The mostly chronically sensitive species (Rainbow trout) was tested in chronic exposure with 90-d NOEC = 0.00213 mg a.s./L and EC10 = 0.0024 mg a.s./L. On the basis of the lowest endpoint and the substance in not rapidly degradable, quinoclamine should be classified as: Chronic category 1. The endpoint being in the range 0.001 – 0.01 mg/L, the chronic M-factor is 10.

We note that the conclusion on classification for quinoclamine, based on the above mentioned key studies is the same as that proposed by the dossier submitter.

Dossier Submitter's Response

It is acknowledged that the use of formulation data for classification purposes may need further discussion. We proposed to use formulation studies as key data when no reliable corresponding study is available with the active ingredient. In the case of algae, the most sensitive value was derived from a formulation study with Scenedesmus subspicatus. The available active ingredient study on the same species did not fulfil the validity criteria, however, the results supported that this species is more sensitive than the second tested species with the active ingredient (Navicula). There are no indications from available data that the formulation has a significantly different toxicity profile compared to the active ingredient.
Therefore, in this case we think it is justified to use the reliable endpoint from the formulation study as a surrogate for the active ingredient. Note that in this case the margin to the next M-factor level is high, which implies a relatively high certainty that any co-formulant would not influence the overall classification proposal.

Discussion may also be needed on selection of key study for aquatic macrophytes. It is agreed that Lemna is the most commonly used species, however, from our view the most sensitive species from each taxonomic group should be used for classification purposes. It should be noted though, that this issue would not change the overall conclusion since the Chronic classification rely on the fish data.
RAC's response
Acute aquatic hazard RAC agrees to the comments made by the commenting MS. Chronic aquatic hazard RAC agrees to the conclusion on biodegradation and bioaccumulation. Selection of key data for aquatic plants RAC notes that the test guideline used in the Myriophyllum test was a sediment-free OECD TG 238 test. The test duration does not allow multiple generations as normally required from a chronic test. However, as the substance is a herbicide and had severe effect in the test, RAC is of the opinion that the data can be considered both acute and chronic.