Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Margosa, ext. [from the kernels of *Azadirachta indica* extracted with water and further processed with organic solvents]

EC Number: 283-644-7
CAS Number: 84696-25-3

CLH-O-0000006926-62-01/F

Adopted

10 December 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON MARGOSA, EXT. [FROM THE KERNELS OF AZADIRACHTA INDICAEXTRACTED WITH WATER AND FURTHER PROCESSED WITH ORGANIC SOLVENTS]

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: Margosa, ext. [from the kernels of Azadirachta indicaextracted with water and further processed with organic solvents]
EC number: 283-644-7
CAS number: 84696-25-3
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Germany	Trifolio-M GmbH	Company-Manufacturer	1
Comment received				
The differences in timing between biocidal and PPP a.i. CLH procedures are hard to understand and considerable legal uncertainties will remain, because NeemAzal technical is still approved under the name Azadirachtin in PPP regulation until 2024 without the respective classification. We are wondering, how PPP authorities might deal with a cleft situation that will be created, when the same a.i. will be classified under the name Margosa extract as an outcome of the CLH procedure, and what consequences that might have. When the CLH process will result in a classification in PPP processes for Trifolio extract only, this will cause a certain "disharmony" and might be at least an infringement of EU competition law. Therefore the CLH process of the three Azadirachtin-Margosa-substances shall be synchronised as they follow the same timelines in the PPP and biocidal approval process.				
Dossier Submitter's Response				
As the definitions of the active substances "azadirachtin" in the PPP process and "Margosa, ext. [from the kernels of Azadirachta indicaextracted with water and further processed with organic solvents]" in the BP process are based on different criteria, they are formally different substances. The substance definition for azadirachtin will be reviewed within the PPP renewal procedure and, if necessary, adapted to make it compatible with the substance definition in the BP process.				

During the transition period, different classifications may therefore result for formally different substances, which may however be chemically identical.
RAC's response
RAC agrees with the DS's response. Further it should be pointed out that it is RAC's task to evaluate the data submitted in the CLH reports. The present CLH proposal clearly defines which substance is covered (regarding the substance identity). The resulting RAC opinion will cover this specific substance as specified in the CLH report.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Germany	Trifolio-M GmbH	Company-Manufacturer	2
Comment received				
The differences in timing between biocidal and PPP a.i. CLH procedures are hard to understand and considerable legal uncertainties will remain, because NeemAzal technical is still approved under the name Azadirachtin in PPP regulation until 2024 without the respective classification. We are wondering, how PPP authorities might deal with a cleft situation that will be created, when the same a.i. will be classified under the name Margosa extract as an outcome of the CLH procedure, and what consequences that might have. When the CLH process will result in a classification in PPP processes for Trifolio extract only, this will cause a certain "disharmony" and might be at least an infringement of EU competition law. Therefore the CLH process of the three Azadirachtin-Margosa-substances shall be synchronised as they follow the same timelines in the PPP and biocidal approval process.				
Dossier Submitter's Response				
See comment 1				
RAC's response				
RAC agrees with the DS's response and and refers to its response to comment number 1.				

Date	Country	Organisation	Type of Organisation	Comment number
22.01.2020	Germany		Individual	3
Comment received				
The description of history of the previous classification and labelling in Part A (2.1.) has been cut very short and some facts concerning the renewal date for the PPP active substances (Part B 1.1.) are not correct. Therefore uncertainties remain on the classification of the active substances used in PPP and RAC might consider it appropriate to postpone the harmonized classification of the Margosa Extract with water as outlined in the following: In 2014 a CLH report for the Neem extract of Trifolio and two other sources has been published under the name Azadirachtin. Likewise the plant protection a.s. authorisation, this report comprised three Neem seed extracts and classified all the same. A separate CLH-report was published for Margosa ext. (name of the active substance chosen by authorities during the biocidal registration process). We must bear in mind that Azadirachtin from Trifolio source (PPP active substance) and Margosa ext. from Trifolio-source (biocidal active substance) are identical. Both a.i.´s are in fact "NeemAzal technical" as the extract was originally named by our company and is still known by our customers. In order to differ between the a.i´s from various sources and to harmonize the name of active substances in plant protection and biocide regulatory work the CLH-proposals were withdrawn after one year. As a consequence four different Margosa				

extracts have been identified. But CLH-dossiers have been submitted for only two extracts until now. Beside this one, a CLH report on Margosa extract (cold-pressed oil of Azadirachta indica seeds without shells extracted with super-critical carbon dioxide) has been published in December 2016. Both are active substances in the meaning of the Regulation EU No. 528/2012. The other two extracts are active substances under regulation 1107/2009 and the CLH proposals are expected to be submitted in the framework of the PPP renewal. For the PPP renewal the former a.i "Azadirachtin" (dossier submitted by Azadirachtin taskforce) will be divided in different extracts with separate dossiers. The Trifolio extract (Margosa ext. with water and further processed with organic solvents) is one of those three extracts and subject to both regulations (PPP and Biocide). According to SANTE-2016-10616–rev 9 a new expiring date for Azadirachtin has been set at 31.05.2024 instead of 2021 as mentioned by the CLH submitter. The biocidal active substance (Trifolio source) expires at 30.04.2024 - thus, the active substance Azadirachtin (comprised of three Margosa extracts) and the one biocidal Margosa extract will expire nearly at the same time. Although Trifolio extract is subject to both regulative frameworks, the justification for the CLH proposal is based on its biocidal use only. We have to consider that the Trifolio extract is still approved under the name Azadirachtin as a plant protection active substance as are the other two extracts which are still not submitted to harmonized classification. Separate harmonized classification for the three plant protection extracts is still pending and the classification of the biocidal Margosa extract cannot be transferred to the active substance Azadirachtin as long as the tree extracts are still approved as one active substance under the PPP regulation. Consequently the outcome of the classification process should not be relevant for the classification of Azadirachtin (Trifolio source included) in the current stage of approval. Therefore RAC might consider to postpone the harmonized classification for Margosa Extract with water until renewal data will be submitted (plant protection and biocidal active substances) as there might be new studies which could clarify uncertainties which occur in the present dossier concerning the aquatic and reproductive classification
Dossier Submitter's Response
We agree that the situation is complex. There are various extracts, some of which are grouped together under the name of "azadirachtin". Other extracts are considered individually. However, this CLH proposal aims to harmonise the classification of a well-defined extract - namely "Margosa, ext. [from the kernels of Azadirachta indicaextracted with water and further processed with organic solvents]". Effects on the biocide or PPP process or product authorisation are not the subject of this procedure. During the transition period, different classifications may therefore result for formally different substances, which may however be chemically identical.
RAC's response
RAC agrees with the DS's response and referse to its response to comment number 1.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
21.01.2020	Germany	Fördergemeinschaft Ökologischer Obstbau e.V. (FÖKO)	National NGO	4

Comment received

We are inclined to comment the classification of the Margosa-extract as developmental toxicant (Repr. 2; H361d) (Short summary, page 9)
To our knowledge, this is a case where the decision on the classification is not clear from the relevant data set and therefore experts need to decide considering the weight of evidence.
In this context, it needs to be considered that the data set was already presented for the registration of Azadirachtin as active substance in plant protection products in the peer review of the pesticide risk assessment and there is a report of EFSA (EFSA Journal 2011;9(3):1858). In the active substance authorisation under EU Regulation 1107/2009 it was clearly discussed that there was no weight of evidence that would justify such a classification.
Furthermore, the Margosa extract of Terra Nostra already passed the Risk Assessment and was not classified for reproductive toxicity, based on a developmental study on rabbits with dermal application, whereas due to the absence of data not all risks with respect to reproductive toxicity could be addressed. (CLH report, Dec. 2016).
Since the seed extracts of Azadirachta indica are highly popular, traditional botanicals used for multiple purposes, including medicinal and cosmetic ones, over hundreds of years without any evidence that the use could lead to damage to the unborn child we urge the Committee for Risk Assessment to consider very carefully if the weight of evidence of the data set does really justify such a classification.

Dossier Submitter's Response

See response to comment number 5 (same as comment number 4 with further elaboration).

RAC's response

RAC agrees with the response provided by the DS. In addition RAC wants to point out that although neem tree extracts are highly popular traditional botanicals and have been used for multiple purposes over hundreds of years, without any evidence that the use could lead to damage to the unborn child, no reliable epidemiological study was provided, that would allow a thorough assessment of developmental effects of these extracts in humans. The fact that the mentioned extracts are considered to be rather diverse regarding their composition (depending on source material as well as extraction method applied) further complicates an assessment of potential effects of these extracts on humans.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Germany	Bund Ökologische Lebensmittelwirtschaft e.V. (BÖLW)	Industry or trade association	5

Comment received

We are inclined to comment the classification of the Margosa-extract as developmental toxicant (Repr. 2; H361d) (Short summary, page 9)
To our knowledge, this is a case where the decision on the classification is not clear from

the relevant data set and therefore experts need to decide considering the weight of evidence.
In this context, it needs to be considered that the data set was already presented for the registration of azadirachtin as active substance in plant protection products in the peer review of the pesticide risk assessment and there is a report of EFSA (EFSA Journal 2011;9(3):1858). In the active substance authorisation under EU Regulation 1107/2009 it was clearly discussed that there was no weight of evidence that would justify such a classification.
In its "Specifications and Evaluations for Agricultural Pesticides – Azadirachtin" the UN Food and Agriculture Organisation (FAO) concludes its summary of azadirachtin's toxicological profile as follows: "The sub-acute to chronicoral toxicity of azadirachtin/NeemAzal© TK is relatively low. Azadirachtin was not carcinogenic in rats after administration via the diet, and did not lead to any malformations in rats and their offspring." Part of FAO's summary is also EPAs assessment of the study in question in the CLH report (EIP 2/952493: 1997. A study of developmental toxicity in rats (gavage administration)). This toxicological assessment is further mirrored in EPA's overall conclusion regarding human health risks through azadirachtin: "Under the registration and reregistration process, the Agency conducted a complete toxicological risk assessment and it can be determined that pesticide products containing Azadirachtin as an active ingredient are not expected to cause unreasonable risks to human health (U.S. EPA, 2015 and U.S. EPA, 2009c)."
Furthermore, the Margosa extract of Terra Nostra already passed the Risk Assessment and was not classified for reproductive toxicity, based on a developmental study on rabbits with dermal application, whereas due to the absence of data not all risks with respect to reproductive toxicity could be addressed. (CLH report, Dec. 2016).
Finally, the seed extracts of azadirachta indica are highly popular, traditional botanicals used for multiple purposes, including medicinal and cosmetic ones, over hundreds of years without any evidence that the use could lead to damage to the unborn child. As outlined above the pesticide active substance registration risk assessments in the EU as well as in the USA using the same data did not draw the conclusions stated in this report. We therefore urge the Committee for Risk Assessment to consider very carefully if the weight of evidence of the data set does really justify such a classification.

Dossier Submitter's Response

Existing assessment reports by other authorities, e.g. by EFSA, US EPA, FAO, can be used for identifying the available information, but the assessment for harmonised classification and labelling purposes should be undertaken independent of the outcomes of the assessments by other authorities. This procedure pertains to hazard identification and harmonised classification, whereas the other assessments cover the entire risk assessment approach including exposure assessment, which is not within the scope of the classification process.

The justification for the Repr. 2 H361d classification is mainly based on the heart malformations and anomalies observed in the developmental toxicity study from 1997 (performed in accordance with OECD Guideline 414) in rats, and these effects might not be secondary to maternal toxicity. There appears to be a dose-dependent trend in the severity of these effects, e.g. small interventricular septal defects as anomalies observed at the mid-dose leading to multiple heart malformations and anomalies observed at the high dose.

We acknowledge the uncertainties in the current dataset for developmental toxicity potential of Margosa extract, which are already mentioned in the CLH report, but the existing data also does not allow drawing the conclusion that these effects are not

substance-related. The experimental design of other studies on reproductive toxicity (e.g. 2-generation reproduction studies) are not directly comparable with that of developmental toxicity study, e.g. OECD Guideline 414. Furthermore, it is worth mentioning that the substances tested in the other reproductive/developmental toxicity studies differ significantly in composition, e.g. containing a lower percentage of azadirachtin (~8-12 %) than the test substance used in the key rat developmental toxicity study from 1997 (36.7 % azadirachtin A).

Overall, expert judgment is required, and it is our opinion that more weight needs to be placed on the heart effects observed in the developmental toxicity study than the lack of effects observed in other reproductive studies of different experimental design and test substances. Considering the existing information altogether, the proposed classification of Repr. 2 H361d is considered justified.

RAC's response

RAC agrees with the response provided by the DS. In addition RAC wants to point out that the available 2-generation studies are not only insufficient with regard to its test design to fully assess the potential developmental toxicity, but also the applied doses were too low to assess the reproductive toxicity of *Margosa Extract with water*.

Date	Country	Organisation	Type of Organisation	Comment number
10.12.2019	Netherlands	<confidential>	Company-Importer	6

Comment received

Nufarm considers the classification proposal as Repr. 2 H361d not appropriate for NeemAzal technical as a.s.
During the EFSA peer review process of the NeemAzal technical a.s. it was concluded that it was not appropriate to classify the a.s. as R63 based on the dataset available and incidences seen in rat studies. The majority of the MS agreed not to propose any classification.
Effects in rats was only seen in one study (1997) and only in one test animal above the accepted historical control data which is statistically insignificant.
In no other study/report effects on rat where observed.
Moreover the discussed effect was observed in the highest test dose only where other toxic effects cannot be excluded.
NeemAzal technical a.s. is an effective biological a.s., which is of high value for the farmers in current times of continuously decreasing number of chemical alternatives. A classification as Repr. 2 H361d would not only result in loss of value of this biological product, but would lead to a shift to chemical alternatives which might be more harmful than NeemAzal technical a.s.

Dossier Submitter's Response

It should be clarified that the majority of the experts at the EFSA peer review meeting agreed not proposing any classification because this is the remit of the ECHA's RAC and not of EFSA.

In the rat developmental toxicity study (1997) that is key for the proposed classification, malformations and variations associated with the heart were observed in foetuses from various litters of the high and mid-dose groups. In particular, 3 foetuses of different litters at the mid-dose exhibited small interventricular septal defects (anomalies), and 1 foetus at the same dose exhibited severe interventricular septal defect and a malrotated heart (both malformations). At the high dose, these effects persisted and 2 foetuses from

different litters also exhibited duplicated inferior vena cava. Even though the incidences are low, there appears to have some dose-dependent trend in increasing severity. At the mid-dose, the dams did not exhibit adverse maternal toxicity (some post-dosing salivation on isolated occasions between days 17 and 19 *post coitum* and transient decrease in food consumption). Taking this altogether, the data suggest that, even though the incidences were low, developmental effects could be already present prior to overt maternal toxicity.

We acknowledge the absence of these findings in the other reproductive toxicity studies in rats. However, the outcomes of the 2-generation reproductive toxicity studies should not be directly compared with that of the developmental toxicity study studies, considering the differences in the dosing paradigm (e.g. in the developmental toxicity study, pregnant dams were exposed to the test substance at a critical development period of organogenesis, which is different than the multiple week-exposure starting before mating in the 2-generation reproductive toxicity study).

It should be emphasised that considering the severity of the effects (heart malformations and anomalies) and referring to the CLP Guidance, Annex I: 3.7.2.4.1, "*adverse effects in the embryo/foetus shall be first considered, and then maternal toxicity,…to help reach a conclusion about classification.*" is worth mentioning that the experts at the EFSA peer review meeting supported this argumentation but refrained from classification proposal due to the reason explained above in this response.

RAC's response

RAC agrees with the response provided by the DS. RAC is further of the view that there was no evidence for maternal toxicity at the mid-dose and there was only insignificant maternal toxicity at the top-dose. RAC does not consider the observed heart related malformations and variation to be secondary to maternal toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Germany	Trifolio-M GmbH	Company-Manufacturer	7

Comment received

Margosa Extract with water (NeemAzal® technical) is also registered in USA under the name Azadirachtin. The renewal of the active substance has already started and an Interim Report has been published in September 2019 (EPA-HQ-OPP-2008-0632 www.regulations.gov). Although only a short summary of the human health evaluation has been published, we want to point out that EPA conducted a complete risk assessment and comes to the conclusion that no unreasonable risk to human health is expected:

"The toxicological database is considered complete for characterizing hazard and assessing risk from the active ingredient Azadirachtin. All risk assessment and data needs listed in the Agency's regulations and scientific policies. Under the registration and reregistration Azadirachtin Final Work Plan, including human health risk assessments, have been fulfilled. No additional studies are anticipated to be needed for this registration review. All data requirements, per 40 CFD 158.2050, have been fulfilled for Azadirachtin. Hazard and exposure data, Agency risk assessments, and other information on this active ingredient were evaluated against standards established by FIFRA and process, the Agency conducted a complete toxicological risk assessment and it can be determined that pesticide products containing Azadirachtin as an active ingredient are not expected to cause unreasonable risks to human health" (Cited from the Azadirachtin Proposed Interim Registration Review Decision Case Number 6021)

Dossier Submitter's Response
See response to comment number 8 (same text as comment number 7).
RAC's response
RAC agrees with the response provided by the DS

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Germany	Trifolio-M GmbH	Company-Manufacturer	8
Comment received				
Margosa Extract with water (NeemAzal® technical) is also registered in USA under the name Azadirachtin. The renewal of the active substance has already started and an Interim Report has been published in September 2019 (EPA-HQ-OPP-2008-0632 www.regulations.gov). Although only a short summary of the human health evaluation has been published, we want to point out that EPA conducted a complete risk assessment and comes to the conclusion that no unreasonable risk to human health is expected: "The toxicological database is considered complete for characterizing hazard and assessing risk from the active ingredient Azadirachtin. All risk assessment and data needs listed in the Agency's regulations and scientific policies. Under the registration and reregistration Azadirachtin Final Work Plan, including human health risk assessments, have been fulfilled. No additional studies are anticipated to be needed for this registration review. All data requirements, per 40 CFD 158.2050, have been fulfilled for Azadirachtin. Hazard and exposure data, Agency risk assessments, and other information on this active ingredient were evaluated against standards established by FIFRA and process, the Agency conducted a complete toxicological risk assessment and it can be determined that pesticide products containing Azadirachtin as an active ingredient are not expected to cause unreasonable risks to human health" (Cited from the Azadirachtin Proposed Interim Registration Review Decision Case Number 6021)				
Dossier Submitter's Response				
The complete risk assessment conducted by the US EPA has not been made publicly available as of March 2020 for further consideration, but nevertheless, the outcome of the US EPA assessment (for the purpose of registration of the active substance) should not be extrapolated for the hazard assessment and identification for classification and labelling purposes.				
RAC's response				
RAC agrees with the response provided by the DS.				

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	France	<confidential>	Company-Downstream user	9
Comment received				
This comment deals with the classification proposal initiated by the BAuA (Germany) of the Active Substance "Margosa, ext. [from the kernels of Azadirachta indica extracted with water and further processed with organic solvents]" as Repr. 2; H361d (developmental toxicity) and more especially with the conclusions of the section 4.10 of the related CLH report. Classification proposal is based on partial results of a study performed in 1997 where one				

litter was affected in the mid dose group. All the other studies valid or invalid, conducted on this material or similar material, concluded that there was no evidence of a reproductive effect.

It must be noted that this Active Substance is used for both Biocidal Products and Plant Protection Products (under the name of Azadirachtin). Therefore, it has been already largely investigated in the past within the scope of both BPR and PPPR and notably by the EFSA under the PPPR. It then should be noted that the developmental toxicity and the study performed in 1997 has already been discussed in the past, notably, during EFSA peer review process for the Azadirachtin. It must be highlighted that during this peer review process, MS / experts agreed not to propose any classification as far as the developmental toxicity is concerned. The conclusion was that: "There was a feeling that R63 was not appropriate based on the dataset available and incidences seen in the rat studies. […] Experts voted on the classification issue and a majority agreed to not propose any classification".

It must also be noted, as stated above, that this effect was noticed in no other study or study part conducted on this material or similar material. Even on the quoted study, the effect was noticed only on one litter in the mid dose group. Regarding this last point, it cannot be ruled out that this single result may be an artifact, statistically insignificant, and that the observed effect was not triggered by the product directly but by an external independent factor, as a consequence, said result should be considered as a non-significant and invalid result. A new study could be done to confirm the non-significance of this single result.

Moreover, after further investigation, it should be noted that other studies not listed in the CLH report confirms the inappropriateness of the classification proposal as far as the developmental toxicity is concerned. These additional studies are listed in a confidential annex attached to these comments.

Furthermore, it must also be noted that this Active Substance has been widely, traditionally and safely used for many years now for Plant Protection Products and Biocidal Products for professional but also non-professional users, in many type of products and treated articles and that, as far as we know, no adverse effect have been reported or noticed.

Based on this comments, we strongly believe that a classification of the the Active Substance "Margosa, ext. [from the kernels of Azadirachta indica extracted with water and further processed with organic solvents]" as Repr. 2; H361d is not appropriate. An additional study could also be performed to confirm the harmlessness of the Active Substance.

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment CLH proposal comments annex - confidential - 24-01-2020.pdf

Dossier Submitter's Response

As addressed in the earlier comments, the majority of the experts at the EFSA peer review meeting agreed not proposing any classification because this is the remit of the ECHA's RAC and not of EFSA.

The other studies not listed in the CLH report were already reviewed by us in the past. Below is the summary of the overall evaluation of these studies in regards to the proposed classification:

- The key rat developmental toxicity study (in accordance with OECD Guideline 414) with foetal heart malformations and anomalies observed in 2 doses was conducted with the test substance NeemAzam Technical containing 36.7 % azadirachtin A.
- The other developmental toxicity studies (e.g. as published by Srivastava & Raizada, 2001, 2007) tested Margosa extract with significantly different compositions than the NeemAzam Technical tested in the abovementioned key study. In particular, in the Srivastava & Raizada publications, azadirachtin technical 12 % was tested, and the doses were lower (tested up to 1000 ppm, equivalent to 50 mg/kg bw) than the one tested in the key study. Therefore, the findings from the other developmental toxicity studies cannot be directly compared with that of the abovementioned key rat developmental toxicity study.
- Due to the difference in exposure paradigms, strain/species tested, health statuses of the animals (i.e. non-pregnant vs. pregnant animals exposed) and/or endpoints measured, results from the 2-generation reproductive studies (some of which are included in the CLH report) should not be directly compared with that from a developmental toxicity study. We acknowledge the uncertainty regarding the overall dataset for developmental toxicity, but there is no robust evidence to exclude the potential rat developmental effects in the heart after *in utero* exposure to Margosa extract.
- It is worth mentioning that in the scientific publication by Dallaqua et al. (2013), treatment with neem seed oil (one potential source of *Azadirachta indica* and thus Margosa extract) during pregnancy caused abnormalities (malformation and variation) in rat foetuses, whereas treatment of *Azadirachta indica* (azadirachtin at 1.0 mg/mL/day) alone during pregnancy did not. This suggest that azadirachtin might not be responsible for the malformations, but it is not clear if other components in neem seed or Margosa extract could be responsible for the developmental effects.

The safe use of the substance, which is more relevant for risk management procedure than for risk assessment, does not play a role on the classification process of Margosa extract.

To summarise, the Repr. 2 H361d classification is proposed because of the observed heart effects (anomalies and malformations) in the developmental toxicity rat study, which might not be secondary to maternal toxicity and show a dose-related trend in increasing severity. The lack of comparable data among different sources of the substance with different compositions or studies with similar experimental design precludes drawing the conclusion that the foetal effects in the heart after *in utero* exposure are not substance-related. Given the severity of the effects and the existing uncertainties, the proposed Repr. 2 H361d classification is considered justified.

RAC's response

RAC agrees with the response provided by the DS and refers to its response to comment number 4.

Date	Country	Organisation	Type of Organisation	Comment number
09.12.2019	Germany	Trifolio-M GmbH	Company-Manufacturer	10

Comment received

We, Trifolio-M GmbH are undoubtedly convinced that Repr. 2; H361d classification of our refined neem kernel extract, nowadays called "Margosa, ext. [from the kernels of Azadirachta indica extracted with water and further processed with organic solvents]", is not appropriate.

However, in the case that we have to accept this classification, based on a conclusion by the authorities that differs from our conviction, we think specific concentration limits (SCLs) above the generic concentration limits (GCLs) must be set for the substance via the CLH procedure.
As presented in the attached statement (Statement SCLs_Reprotox_Margosa ext. DEZ19), Margosa, ext. should be associate with the low potency group, referring to an ED10 ≥400 mg/kg bw/day. In consequence, SCL´s between 3 % and 10 % must be applied in order to achieve an appropriate classification of the related products.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Statement SCLs_Reprotox_Margosa ext. DEZ19.pdf

Dossier Submitter's Response

The current dataset for developmental toxicity is not robust or adequate enough to set a specific concentration limit (SCL). The proposed Repr. 2 H361d classification is primarily based on the rat developmental toxicity OECD Guideline 414 study using one source of Margosa extract (NeemAzem Technical with 36.7 % azadirachtin A), showing limited indications of foetal heart malformations and anomalies at 2 doses (dose-dependent trend in increasing severity and effects might not be secondary to maternal toxicity).

The CLP Guidance (version 5.0, July 2017), Section 3.7.2.6.2, states that "*if the classification of a substance in Category 2 is done on the basis of 'limited evidence', the quality of the available data will in such cases determine whether a potency assessment is possible. In cases where no further evaluation is possible, the generic concentration limits* [GCL] *of CLP apply.*"

In our opinion, it is not possible to perform a potency assessment using one OECD Guideline 414 study of one source of Margosa extract. The combined incidence of heart malformations and anomalies is low with 4/306 foetuses from 4/23 litters (1.3 % and 17.4 %, respectively) affected at the mid-dose of 225 mg/kg bw/d and 5/308 foetuses from 5/23 litters (1.6 % and 21.7 %, respectively) affected at the high dose of 1000 mg/kg bw/d (refer to Table 36 of CLH report). If ED_{10} is determined based on incidence in pups, the value would be above 1000 mg/kg bw/d. However, if the value is determined based on incidence in litters, the ED_{10} would be below 225 mg/kg bw/d (around the medium potency range). No further scientific evidence is available to determine which parameter would be more appropriate, and therefore, it is not possible to estimate an ED_{10} for the heart effects (see CLP Guidance, Section 3.7.2.6.3.2).

Considering the effect levels (potentially starting in the medium potency range) and the existing data, the database is not adequate enough to deviate from the GCL and set a SCL with confidence.

RAC's response

RAC ist of the view that in the present case the available study appears reliable to assess the potency of the test material in this study. The low incidences of malformations observed are considered to represent the limited evidence supporting classification in category 2.
As explained by the DS, an ED10 value above 1000 mg/kg bw can be derived when based on incidence in pups, while an ED10 below 225 mg/kg bw is achieved when based on litter incidence (considering both malformationas and anomalies together).
In section 3.7.2.6.5 of the CLP guidance several modifying factors are listed, which should be considered when deciding whether SCLs should be applied in specific cases. Upon

comparison of the data available for *Margosa Extract with water* with the modifying factors RAC concludes that *Margosa Extract with water* should remain in the medium potency group and the general concentration limit of 3% should be applied.

Date	Country	Organisation	Type of Organisation	Comment number
09.12.2019	Germany	Trifolio-M GmbH	Company-Manufacturer	11

Comment received

Since the launch of the first CLH proposal on the botanical active substances, extracted from the seeds of the tropical Neem tree Azadirachta indica Juss., in 2013, Trifolio-M has tried to prevent the classification of its extract with H361d, because we are convinced it is not justified. In the course of the BP process we received the comment, that it is a borderline case and we should use the Public Consultation.
Therefore, we strongly request to revise the proposed classification and present attached a statement (Margosa statement dev tox GAB NOV2019), in which all facts are examined in detail, finding insufficient evidence for classification, likewise the experts during the PRAPeR Expert Meeting 79, when Azadirachtin was discussed for inclusion on the List of active substances approved for use in plant protection in 2010. Considering all information in accordance with the CLP guidelines, applying a weight of evidence approach, no classification for reprotoxicity is warranted.

In "United Nations Economic Commission for Europe (UNECE), 2004. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Part 3", the weight of evidence is postulated as follows: "Classification as a reproductive toxicant is made on the basis of an assessment of the total weight of evidence. [...] Both positive and negative results are assembled together into a weight of evidence determination. However, a single, positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification".
In the present case of the Trifolio-source of Margosa ext., the reprotox-study on rats considered for this classification, must be regarded as a borderline case and statistically significant results are lacking. Conspicuous features occur at high dosage only and in presence of maternal toxicity. It seems hardly appropriate to draw conclusion on the outcome of this single study without consideration of the biological significance, influence of maternal toxicity and consistency of the developmental findings in other studies.
We acknowledge the efforts of European Authorities towards consumer and environmental protection. However, when a substance with a long history of safe use, but a complex composition and therefore challenging in data provision alongside the catalogue of OECD standards developed for synthetic chemical substances, will be stigmatized as "suspect of damaging the unborn child" while there are arguments that remained discounted, we wish for a cautious reconsideration.
All additionally available information should be considered in a weight of evidence-approach. Therefore, the following aspects must be taken into account and are discussed more detailed in the statement attached:
• further studies with other Neem-Extracts,
• historical control data
• maternal toxicity
• no evidence for reprotoxic mode of action
• long background of safe use

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Statements Trifolio-M H361d_H410.zip

Dossier Submitter's Response
We agree that expert judgment and a weight-of-evidence approach need to be taken for drawing the conclusion of the Repr. 2 H361d classification. In our opinion, there is not sufficient evidence to conclude that the heart effects observed in 2 doses (225 and 1000 mg/kg bw/d) tested in the developmental toxicity study are not substance-related, and therefore, the proposed Repr. 2 H361d classification is considered justified. The Neem extracts tested in other developmental toxicity studies, which observed no adverse developmental effects, have significanty different compositions than the one tested in the developmental toxicity study with observed heart effects. In particular, the tested substance (NeemAzal Technical) in the 1997 developmental toxicity study in rats with observed heart effects (in accordance with OECD Guideline 414) contained 36.7 % azadirachtin A, whereas the other Neem Extract tested in a similar developmental toxicity study (i.e. also in accordance with OECD Guideline 414) contained only a total of 8.5 % (w/w) azadirachtin A and B. Other developmental toxicity rat studies, e.g. as published by Srivastava & Raizada, 2001 and 2007, tested substances also with lower percentage of azadirachtin (12 %). There are no other developmental toxicity studies with Margosa extract (e.g. with similar specification as NeemAzal Technical) that are comparable to that of the abovementioned 1997 developmental toxicity study; thus, this study is considered as key data in the classification process. It is worth mentioning that in the scientific publication by Dallaqua et al. (2013), neem seed oil (one potential source of *Azadirachta indica* and thus Margosa extract) treatment administered during pregnancy caused abnormalities (malformation and variation) in rat foetuses, whereas administration of *Azadirachta indica* (azadirachtin at 1.0 mg/mL/day) alone during pregnancy did not. This suggest that azadirachtin might not be responsible for the malformations, but it is not clear if other components in neem seed or Margosa extract could be responsible for the developmental effects. In the key rat developmental toxicity study (1997), foetal heart malformations and anomalies were observed at both mid- and high doses, and these might not be secondary to maternal toxicity. We disagree with the commenter in their statement that the mid-dose (225 mg/kg bw/d) is considered as a maternally toxic dose. In the review by Beyer et al., 2011 as cited by the commenter, marked maternal toxicity is characterised by "*decreased body weight gains of greater than 20 % for prolonged periods*". In this case, there was a non-statistically significant reduction in body weight gain of -18 % (8.5 g/rat with the mid-dose vs. 10.4 g/rat with the control) observed only at the beginning of treatment from gestation days 6-8, which might be related to the slight decrease in food intake at the same period. This finding, along with lack of adverse effects observed, is not sufficient to consider this mid-dose as maternally toxic. The additional historical control data provided for the foetal heart effects are not appropriate for comparison as these data originated from different laboratories with different animal sources. Due to the difference in exposure paradigms, strain/species tested, health statuses of the animals (i.e. non-pregnant vs. pregnant animals exposed) and/or endpoints measured, results from the 2-generation reproductive studies or repeated dose toxicity studies should not be directly compared with that from a developmental toxicity study. We acknowledge the uncertainty regarding the mode of action leading to developmental toxicity, but there is no robust evidence to exclude the potential rat developmental effects in the heart after *in utero* exposure to Margosa extract.

It should be clarified that the majority of the experts at the EFSA peer review meeting agreed not proposing any classification because this is the remit of the ECHA's RAC and not of EFSA.

Lastly, the safe use of the substance, which is more relevant for risk management procedure than for risk assessment, does not play a role on the classification process of Margosa extract.

Altogether, current data suggest (and cannot disprove) that the heart effects observed in the developmental toxicity study in rats (in accordance with OECD Guideline 414) might be substance-related. Proposed Repr. 2 H361d classification is considered justified.

RAC's response

RAC largely agrees with the response provided by the DS and refers to its response to comment number 4.
Regarding the historical control data provided by Trifolio-M GmbH during public consultation, it should be noted that they were not considered for the first version of the draft opinion and RCOM document, because it was assumed that they originated from a different laboratory than the one that had conducted the study by Anonymous, 1997e, f (i.e. Huntington laboratories). However, Trifolio-M GmbH clarified well before the plenary meeting that the provided historical control data were fomr the same facility. The reason for the confusion was that the data were provided by Envigo, the successor institute of Huntington. An in depth analysis of the provided data was carried out by RAC and included in the second version of the draft opinion before the plenary discussion. The provided data consist of 24 studies conducted between July 1994 and February 1997. These data included 11 studies that had already been considered by the DS (1994 - 1995). In these 24 studies interventricular septal defect (malformation) was seen in 4 studies, in 3 of them a single foetus showed the effect, in 1 study 2 foetuses of 2 litters had the effect. No incidence of malrotated heart was seen in any of the 24 studies. Duplicated vena cava was seen in 1 foetus of the 24 studies. No data were presented for the other malformations (i.e. malformed systemic / pulmonary arteries, atrial septal defect with narrow pulmonary vein).
Based on these HCD provided during public consultation (Huntington, 1994 – 1997) the historical incidences for interventricular septal defect were only exceeded for litters at the top dose. For duplicated inferior vena cava the historical incidences were exceeded in the top dose for foetuses and litters. The observed cases of malrotated heart in mid and top dose (one case each) exceeded the historical controls, as this effect was not seen in any of the 24 studies.
Taking together all observed alterations in this organ system in the foetuses, an increased incidence of heart related effects with dose and a dose-related trend in severity can be observed.

In addition the incidence of supernumerary rib 14 was increased at the top dose. Though they are no malformations but variants and only increased in the top dose concomitant with slight maternal toxicity, they were judged to be relevant findings by the PPP expert group.

The HCD from Huntington, 1994 – 1997 also provided incidences for supernumerary rib 14. In these data it was differentiated between full and short rib 14. In only 1 of the 24 studies full supernumerary rib 14 was seen in 2 foetuses from 1 litter (foetuses: 0 – 1.2%, litters: 4%). Short supernumerary rib was seen in all studies with incidences ranging from 4.5% - 20% in foetuses and 25 - 48% in litters. From the full study report it could not be identified whether the incidences observed in Anonymous (1997f) were for full or short rib or for both effects together. Regarding the relatively rareness of full additional rib 14 it might be concluded that the numbers from Anonymous (1997f) consider either both, incidences of

short and full rib 14 together, or only short rib 14 incidences. Based on the available information no direct comparison with the provided HCD is possible. RAC considers the effect as supportive evidence for classification.

Regarding maternal toxicity RAC agrees with the DS that at the mid-dose there was no evidence for maternal toxicity. RAC is further of the view that there was only insignificant maternal toxicity at the top-dose and overall RAC does not assume the observed heart related malformations and variation to be secondary to maternal toxicity.

Regarding the long background of safe use mentioned by the commenter RAC refers to its response to comment number 4.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
09.12.2019	Germany	Trifolio-M GmbH	Company-Manufacturer	12

Comment received

In the "Proposal for Harmonised Classification and Labelling Substance Name: Margosa, ext." of October 2019, the substance Margosa, ext. [from the kernels of Azadirachta indica extracted with water and further processed with organic solvents] is classified as Aquatic Chronic, Category 1, H410 with an M-Factor of 10 regarding the risks to the environment. Trifolio-M does not completely share this view when looking at the available studies.
The classification has been triggered by the lowest long-term effect value (28d-NOEC). This was found for the midge larvae Chironomus riparius in water-sediment studies according to OECD 219 (spiked water) which have been performed for the EU registration of Azadirachtin, considering different extracts and formulated products.
Two long-term studies with larvae of the midge Chironomus riparius using the active ingredient NeemAzal® = Margosa, ext. (Gonsior, 2008a) and the product NeemAzal®-T/S (Gonsior, 2008b) have been performed. Nominal and actual endpoints have been set by the study director as follows:
NOEC NeemAzal®: 0,0184 mg/l (nominal concentration, Gonsior, 2008a)
NOEC NeemAzal®-T/S: 0,573 mg/l (nominal concentration, Gonsior, 2008b)
0,433 mg/l (actual concentration, Gonsior, 2008b)

Although these actual and nominal endpoints have been set according to OECD 219, the classification of Margosa, ext. as "Aquatic Chronic, Category 1, H410 with an M-Factor of 10" is based on calculated endpoints. Finally the given endpoint results in the NOEC = 0.0075 mg a.s./L and 0.006 mg a.s./L, respectively (the latter converted from the result of the product study).
The poor recovery rates of the lead component in these studies (below the limit of quantification) were the reason, why the actual amount measured was not considered to determine the NOEC. The RMS concluded that the nominal exposure of the chironomids was not given throughout the study and therefore, with reference to SANCO/3268/2001, the endpoints were based on the geometric mean of concentrations measured in the water and porewater instead of the initially measured concentrations as recommended by OECD 219.
Please find attached a statement (Expert Statement Trifolio-M H410) which evaluates the original position during EU review. This evaluation reveals that the reference to SANCO/3268/2001 does not fully justify the use of mean measured over initially measured concentrations in the case of OECD 219. Based on the life cycle of chironomids and the intention of the test system to represent a single exposure event (drift,

drainage), it is considered most reasonable to use the nominal or initially measured concentrations instead of calculating the geometric mean of measured concentrations for the determination of the NOEC. The use of nominal or initially measured instead of geometric mean measured concentrations is also supported by the EFSA technical report (2015, chapter 3.1).
Therefore the crucial endpoint for chronic toxicity classification is the nominal NOEC (28d-NOEC= 0,0184mg Margosa, ext./l) for the midge larvae Chironomus riparius in a water-sediment study according to OECD 219.
Consequently, we do not agree with the M-factor. For substances not fulfilling criteria for rapid degradation, NOEC ≤ 0.1 mg/L is the criterion for classification as H410 with Mchronic = 1 (considering 0.01 mg/L < NOEC < 0.1 mg/L) . Therefore Margosa, ext. should be classified as Aquatic Chronic 1, H410 Mchronic = 1, instead of a chronic multiplication factor Mchronic = 10 (as proposed by RMS in the CLH report).

References
EC (European Commission), 2002. Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC (SANCO/3268/2001) rev.4 final, 17.11.2002, pp. 1–62.
EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924, 62 pp.
Gonsior G. (2008a) Assessment of Side Effects of NeemAzal® on the Larvae of the midge, Chironomus riparius with the Laboratory Test Method, Report-no. 2007135601-ASCr, GLP: yes, Published: no
Gonsior G. (2008b) Assessment of Side Effects of NeemAzal®-T/S on the Larvae of the midge, Chironomus riparius with the Laboratory Test Method, Report-no. 2007135501-ASCr, GLP: yes, Published: no

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Statements Trifolio-M H361d_H410.zip

Dossier Submitter's Response

The NOEC based on mean measured concentrations using LOQ/2 was already agreed in 2012 by EU MS for the assessment of Margosa extract in the BP and PPP assessment. Therefore, we wonder why the arguments presented now were not already provided at that time.
In our opinion, the derivation of the NOEC with mean measured concentration is still applicable. In the following we give a short resonse to the main points raised in the attachement:

- Use of nominal/initial measured conc. vs. mean measured concentraions (reference to EFSA, SANCO and OECD 219):
  EFSA and SANCO decisions are only applicable for the risk assessment of active substances used as plant protection products. Any recommendations from them have to be regarded in conjunction with both exposure and effect assessment.
  The classification and labelling of substances in the frame of the CLP Regulation does not take into consideration any exposure models but focused on the intrinsic hazard of a substance. Therefore, use of nominal/initial measured concentrations in cases where analytical monitoring of the test substance concentration reveals a decrease during the exposure period would underestimate the toxicity of the substance and can therefore not be considered for C&L.
  The statement in OECD 219 that the test system is intended for singular exposure events is therefore also not relevant for C&L. As based on the mode of action of

marogsa extract insects are the most sensitive species and the chironomus studies performed according the OECD 219 are the only tests available with aquatic insects, these studies have to be considered for the hazard assessment. As for classification and labelling only the concentration in the water phase is relevant, as the trigger values are based on aquatic concentrations, spiking of the water phase is under this consideration the most applicable exposure pathway.

- Distribution of Margosa extract between water and sediment phase:
  In both OECD 219 studies with Chironomus (Gonsior 2008a and b) the test substance was spiked to the water phase and the concentrations in water, porewater and sediment were measured on days 0, 7 and 28 for 2 test concentrations and for the control. 1 h after spiking (day 0) the measured concentrations show that the main part of the test substance was in the overlying water and some in the porewater of the sediment. The concentration measured in the porewater indicates that distribution of the test substance in the water-sediment system took place. The concentration in the sediment was < LOQ. Considering the physico-chemical properties of Margosa extract, a significant adsorption to the sediment or to food particles or the test vessels is not expected. Such an adsorption is relevant, as stated in OECD 218 and 219, for substances with a log Kow ≥ 5 or a corresponding adsorption or binding potential. The log Kow of Azadirachtin A is 0.09 - 1.37 thus indicating a low adsorption potential to the sediment. Therefore, it is not remarkable that no Margosa extract / Azadirachtin A could be detected in the sediment.
  After 7 days no test substance could be detected in the test system at all.
  A decrease in test substance concentration was also found in the other available aquatic toxicity studies with fish, daphnids and algae that were performed without sediment. Therefore, it can be concluded that the presence of sediment is not (solely) responsible for the decrease in aquatic test substance concentration due to adsorption. Instead, this decrease can rather be explained by hydrolysis and biodegradation of the test substance. As no measured test substance concentrations in the sediment are available, the only reliable solution is to calculate a mean concentration based on LOQ/2, as recommended in OECD GD 23.

- Calculation of sediment concentration for the top 2 mm sediment layer:
  The approach provided in the statement is no proof for the argument that the decrease in the aquatic phase of the test substance is caused by adsorption to the sediment, as it is just a hypothetic estimation based on assumed values and disregarding any degradation processes in the test system.

RAC's response

RAC agrees with DS response. Moreover the ECHA guidance on CLP foresees that the L(E)C50 and NOEC may be calculated based on the geometric mean concentration of the start and end of test. "Where concentrations at the end of test are below the analytical detection limit, such concentrations shall be considered to be half that detection limit". In conclusion, although RAC noted some uncertenties in the substance behaviour in the experiment media, the calculated values are acceptable to obtain a valid NOEC.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	United Kingdom		MemberState	13

Comment received

Ecotoxicologically relevant constituents

All ecotoxicity tests included in the CLH report used the Margosa extract with water or the relevant formulated biocidal product as the test substance. However, the DS for the CLH proposal stated that azadirachtin A is the constituent mainly responsible for the ecotoxicological effect on the target organisms. This contradicts the section on the Human Health Assessment of the CLH report which states that "the whole extract was considered the toxicologically relevant substance because no toxicological data were available to demonstrate that certain components were responsible for the observed toxicological effects". Though Section A5 of the Biocide Assessment Report summarises the mode of action of the constituent azadirachtin (without differentiating the isomers), the mode of action of the Margosa Extract with water as a whole is not clear from the information in the Biocide Assessment Report (2011) or the CLH report. We note that as the extract contains a mixture of limonoids, it is possibly a combined effect of these that leads to a toxic effect on the target, as well as non-target, organisms. For example, Addendum 08 to the EU pesticide assessment Additional Report on azadirachtin (RMS: Germany, 2018) provides evidence that the constituents nimbin and salannin can have similar antifeeding effects in insects to azadirachtin A. The DS for the Additional Report concluded that several constituents, which are common to both Margosa extract with water and the Margosa extracts considered in the pesticide risk assessment for "azadirachtin", show biological activity, although azadirachtin A is the most effective one. We consider that more information on the relative quantities, fate and ecotoxicity of the other known constituents in Margosa extract with water should be provided and discussed by the DS for the CLH report to prove/disprove that azadirachtin A is the most ecotoxicologically relevant component. To this end, Addendum 08 to the Additional Report of azadirachtin could be referenced for the relative bioactivity of the constituents of the Margosa extract with water.

RMS: Germany, (2018). Addendum 08 to the Additional Report of 10 December 2009 (relating to Volume 3, B.5, B.6, B.7, B.8 and B.9); Confirmatory Information; Azadirachtin; Rev. 12 January 2018. Available: http://registerofquestions.efsa.europa.eu/roqFrontend/outputLoader?output=ON-5234. Last accessed 09/01/2020.

Toxicity to algae

We note the following points about the toxicity of Margosa extract with water to algae, although we envisage that they will not affect the classification.

Only one study on the toxicity of Margosa extract with water to algae/aquatic plants is included in the CLH report. The effect values in this study by Wenzel (2002) are based on nominal concentrations of Margosa extract with water because azadirachtin A was not stable in the test system. Whilst initial measured concentrations of azadirachtin A were between 85 – 113 % of the nominal, this approach is inconsistent with the approach taken for most other endpoints where the effect concentrations were calculated from the mean measured concentrations of azadirachtin A and the content of azadirachtin A in

Margosa Extract with water. For comparability, the effect concentrations for the algal study should ideally also be based on mean measured concentrations of azadirachtin A as a worst-case.

In the same study, the DS for the CLH report highlighted that the control cultures did not follow exponential growth throughout the test and instead a lag phase was observed for the first 24 h. Therefore, the study is strictly not valid, although the DS regards the test as acceptable for the effects assessment because they consider that algae are clearly the least sensitive of the tested aquatic organisms. To support that this study is relevant to the classification, we note that cell concentrations increased by a factor of 54.1 from 0 to 72 h in the controls meeting the validity criteria for control cell concentrations to increase at least by a factor of 16 within three days. However, information on the mean coefficient of variation for section-by-section specific growth rates and the coefficient of variation of average specific growth rates in the control cultures should ideally be supplied by the DS to check whether other relevant validity criteria for control growth were met according to OECD 201. This information is not included in the Biocide Assessment Report or the CLH report. We highlight that although algae and other aquatic plants are not envisaged to be sensitive, no other toxicity data are available for this taxonomic group.

Chronic toxicity to invertebrates

The proposed chronic classification is based on a Chironomus study which used a water-sediment test system. We normally prefer not to use endpoints from studies with sediment in the test systems for classification because the sediment can influence the exposure of the test substance. However, in this instance, we agree that the Chironomus endpoint should be used for classification because exposure was predominantly via the water phase. This is supported by the measured concentrations of azadirachtin A which were below the LOQ in the sediment throughout the duration of the study. Azadirachtin A also has a low sorption potential with Koc values ranging from 20.6 – 65.4 mL/g. Furthermore, Margosa Extract with water has an insecticidal mode of action. Therefore, insects can be expected to be the most sensitive taxonomic group as indicated by the results of the Chironomus studies. Excluding the Chironomus studies from the dataset used for classification could mean that the classification is not protective of aquatic insects as no toxicity data are available for other aquatic insects.

Formulation additives

Ecotoxicity tests with the biocide product NeemAzal-T/S are included in the CLH report. We note that the co-formulants may influence the toxicity, or the bioavailability of the active substance despite not being classified for human health or the environment themselves. We have not reviewed information in the confidential annex of the biocide assessment report to confirm the composition of the formulation, but the RAC may wish this information to be made available to them. This information may be important if the RAC decide not to base the classification on the Chironomus endpoints because the next lowest chronic endpoint is from a Daphnia magna study conducted with the formulated product. The NOEC at 0.1 mg/L (based on the calculated concentration of Margosa extract with water) from this study with Daphnia magna would result in a classification for Aquatic Chronic 1 with an M-factor of 1. By comparison, the NOEC of 1.84 mg/L from the other Daphnia magna study, which was conducted with the active substance, would not result in an Aquatic Chronic classification. The difference between these NOEC values could indicate that the formulation additives in NeemAzal-T/S increase the toxicity of the active substance. In contrast, the NOEC values from the Chironomus studies conducted

with the formulation product compared with the substance (Margosa Extract with water) were in the same concentration range when based on the concentration of Margosa extract with water.

Dossier Submitter's Response

Ecotoxicologically relevant constituents

We agree that there is a discrepancy between the statements in the environmental hazard assessment and human health parts of the CAR. The statement from the human health part is considered to be also relevant for the environmental hazard assessment.

Toxicity to algae

- As reported, in the algae study, not only Azadirachtin A was measured but also Azadrichatin B. As the latter component was found to be stable in the test substance and as it is unclear, which of the components is responsible for the effects observed, the calculation of a mean concentration based on Azadirchatin A is not considered to be appropriate. As the study is anyhow not valid an algae are clearly not the most relevant test species for Marogsa extract, we see no need to derive a mean measured concentration based on Azadirachtin A.
- The mean coefficient of variation for section-by-section specific growth rates in the study was 1.2 % and thus < 7 %, as requested by the guideline. This validity criterion is therefore fulfilled.
- The coefficient of variation of average specific growth rates in the control cultures was 49.58 % and thus significantly above the validity criterion of ≤ 35 %. Therefore, this validity criterion is not fulfilled.
-

Chronic toxicity to invertebrates

Thank you for your support!

Formulation additives

The composition of the biocidal product NeemAzal-T/S is contained in the confidential Appendix to the CAR. The identity of the other components does not indicate that they would increase the toxicity of the active substance. The results from the two long-term daphnia studies are indeed not quite consistent but we could not find a reason for the difference in the NOECs by a factor of 18. On the other hand, as you also stated, the results from the two chironomus studies are in good agreement.

RAC's response

RAC agrees with the DS. In particular regarding the toxicity to algae, although the NOEC for Margosa extract was derived from a lead component different from Azadiracthin A (i.e. Azadiracthin B), it is a known main component and it is measured during the test. Regarding Formulation additives, RAC agrees that the identity of the other components does not indicate that they would increase the toxicity of the active substance, and that results from the two Chironomus studies are in good agreement.

PUBLIC ATTACHMENTS

1. Statement SCLs_Reprotox_Margosa ext. DEZ19.pdf [Please refer to comment No. 10]
2. Statements Trifolio-M H361d_H410.zip [Please refer to comment No. 11, 12]

CONFIDENTIAL ATTACHMENTS

1. CLH proposal comments annex - confidential - 24-01-2020.pdf [Please refer to comment No. 9]