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. [cold-pressed oil of *Azadirachta indica* seeds without shells extracted with super-critical carbon dioxide]

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

CLH-O-0000001412-86-202/F

Adopted

9 March 2018

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON MARGOSA, EXT. [COLD-PRESSED OIL OF *AZADIRACHTA INDICA* SEEDS WITHOUT SHELLS EXTRACTED WITH SUPER-CRITICAL CARBON DIOXIDE]

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: Margosa, ext. [cold-pressed oil of Azadirachta indica seeds without shells extracted with super-critical carbon dioxide]
EC number: 283-644-7
CAS number: 84696-25-3
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	France		MemberState	1
Comment received				
According to the assessment report (January 2017), the substance presents relevant constituents (aflatoxine B1: < 2 µg/kg (0.0000002% w/w) ; aflatoxine B1, B2, G1, G2: < 4 µg/kg (0.0000004% w/w)). This information is not confidential and should have been reported in the point 1.2 of the CLH report.				
Dossier Submitter's Response				
Thank you for comment. This statement is correct based on the assessment report. However based on the time point of writing the CLH report and the draft CAR this information was not included in the LoEP as they were seen as confidential. Additionally as the mentioned aflatoxine can be in the substance (here more precisely are mostly not in the substance) we did not add them at the beginning to the composition information. Based on the comments and disscussions during the peer review process it was agreed to add this information to the assessment report to document the low concentrations of aflatoxins in the CO_2 extract. (The levels are below the lowest maximum levels of 2.0 µg/kg allowed in food (e.g. cereals, dried fruit) according to Reg (EC) No 1881/2006.). The comment has no impact on the proposal for non-classification.				
RAC's response				
The confidential information on the composition of *Margosa Extract* has been submitted by the Dossier Submitter and is available for RAC and is considered in the classification discussion. The comment that *Margosa Extract* also has low contamination of aflatoxins is of interest and is taken into consideration. Aflatoxin contamination has no obvious impact on classification proposal.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	France		MemberState	2
Comment received				
It is written that "As no adverse effects were observed in the 90-day study in rats up to approx.1000 mg/kg bw/day" (page 37). This statement is contrary to the sentence mentioned in the repeated dose toxicity (oral route) page 30: "However, as liver weight increases were above 15 % in both sexes, the effect was considered adverse". In order to avoid confusion, it would be clarified that reversible increases in liver weight were found in the 90-day study but were not considered as adverse because histopathologic correlates were lacking.				
Dossier Submitter's Response				
Thank you for indicating the contradiction. The liver weight changes were regarded as adverse. Hence, the sentence on page 37 is not appropriate and should be read as follows: "In a 90-d rat feeding study with Margosa extract, the top dose of 960 mg/kg bw/d (rounded from 962 mg/kg bw/d) induced an increase in liver weight in males and females, without any histopathological correlates, which was reversible within the 4-week recovery period. However, as liver weight increases were above 15 % in both sexes, the effect was considered adverse". This comment has no impact on the proposal for non-classification.				
RAC's response				
It is appreciated that contradicting statement has been revised by the dossier submitter. The comment has no impact on the carcinogenicity classification proposal.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	France		MemberState	3
Comment received				
It is reported in the CLH report that "anti-fertility (contraceptive and abortive) effects of oils and extracts are reported in studies with various mammalian species including humans (overview e.g Schmutterer H., 2002, The Neem Tree, Mumbai) (page 43). If considered relevant, these data should be more deeply described and discussed in this section.				
Dossier Submitter's Response				
The cases were added for completeness only. Additional information is given as follows: "The following reviews of the open literature on neem products and of animal studies on NeemAzal were added. The results are not applicable to the Margosa, ext. [cold-pressed oil of Azadirachta indica seeds without shells extracted with super-critical carbon dioxide]evaluated under PT19 but were added for documentation that a research in the open literature was performed. Health risks are especially to be expected when ill-defined products of questionable sources are used. Adverse effects are reported in particular following oral intake of large amounts of neem preparations with unknown composition (Niemann, L. et al., In: The Neem Tree. Ed. by Schmutterer H. (2002), Mumbai, published) or with well-defined preparations when ingested accidentally or for suicidal purposes." The comment has no impact on the proposal for non-classification.				
RAC's response				
There are no studies carrid out with *Margosa Extract* (cold pressed oil of Azadirachta indica seeds without shells extracted with super-critical carbon dioxide) to conclude on				

fertility effects. The described fertility effects (Niemann, 2002) cannot be taken into consideration since the neem preparations are considered different from *Margosa Extract*.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	France		MemberState	4
Comment received				
Human information (section 4.11.1.4) It is stated that "poisoning from Margosa Extract up to the limit dose of 2000 mg/kg bw is not to be expected". This statement is based on experimental data in rats; therefore the dose of 2000 mg/kg cannot be directly used to conclude on a lack of poisoning in humans at this dose.				
Dossier Submitter's Response				
You are right, the 2000 mg/kg bw were from animal studies and the dose cannot directly be transferred to humans. However, it indicates no potential for acute toxicity of Margosa CO_2 extract in rats which is supported by human medical observational data from workers. The comment has no impact on the proposal for non-classification.				
RAC's response				
Misleading comment has been clarified by dossier submitter. Based on the presented human data no acute toxicity potential can be deduced therefore data from animal studies are considered for classification proposal. RAC concurs with DS that the comment has no impact on the classification proposal.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	France		MemberState	5
Comment received				
FRCA supports the proposal not to classify Margosa, ext. [cold-pressed oil of Azadirachta indica seeds without shells extracted with super-critical carbon dioxide] for the environment.				
Dossier Submitter's Response				
Thank you for your support. Despite our current CLH proposal it should be discussed at RAC level if rapid degradability can be assumed for margosa extract, taking into account that margosa extract is an UVCB substance, containing non-rapidly degradable components of ecotoxicological relevance such as azadirachtin (please, see also our response to the comments from UK). In case, the whole extract is classified as non-rapidly degradable based on these individual components, this would change the outcome of the classification.				
RAC's response				
Noted.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON MARGOSA, EXT. [COLD-PRESSED OIL OF *AZADIRACHTA INDICA* SEEDS WITHOUT SHELLS EXTRACTED WITH SUPER-CRITICAL CARBON DIOXIDE]

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2017	United Kingdom		MemberState	6

Comment received

1. Even though they all apparently share the same CAS and EC numbers, we understand that this cold-pressed Margosa extract using CO2 (intended for use as a PT 19 repellent biocide) is considered to be a different 'substance' to other extracts mentioned in the Report, including the extract with water and organic solvents for which a draft CLH Report was produced in 2014 (subsequently withdrawn) - which is already approved as a PT 18 insecticidal biocide. We also understand that this is a UVCB extract from plant material and so the precise composition may be unknown and more variable than usual. However, we do feel that, without needing to be confidential, further clarity could be given regarding the expected composition and % w/w ranges of individual key active components in the substance, including the limnoids azadirachtin A and B, nimbin and salannin. As these are insecticidal or insect repellent at differing levels, their concentrations could affect the overall environmental fate, toxicity and classification (see below).

For example, the Human Health Section (no. 4, p17) states that the total content of limonoids is determined to be 2.7 ± 0.4 % w/w, including azadirachtin A, whereas Section 5.1.2.3 on simulation testing states that these compounds account for less that 2% in total, with salannin being predominant. The preceding section on ready biodegradability also states that azadirachtin A+B is present at 0.2 %. Could further clarity please be given therefore, regarding the total percentage of limnoids as well as all the individual ones?

2. Relatively little detail is given in Section 5.1.2.2 on the ready biodegradability test, although it is stated that the whole 'substance' is readily biodegradable, including meeting the 10-day time window criterion. Further reference to the biocidal assessment report for this PT 19 Margosa extract (2017), whilst also stating that the extract is readily biodegradable, questions however whether it should be considered potentially P or vP since the key active components (present at ≥ 0.1% w/w) are not themselves considered degradable. We feel that further deliberation could be given at the RAC as to whether this extract can be considered 'rapidly degradable' according to CLP criteria if key active components are not themselves rapidly degradable. It may be though, that the percentage of the limnoids in this particular extract is relevant here, hence the need for further clarity on this.

3. On the basis of the acute only aquatic toxicity information included in this 2017 CLH report, no aquatic classification has been proposed (Section 5.4-5.6). No chronic classification is proposed based on an algal NOEC, however fish are more acutely sensitive (>10x) and so a surrogate calculation using the fish LC50 could also have been included for completeness.

The previously mentioned 2014 CLH report for a different Margosa extract, as well as the 2014 report for azadirachtin (also withdrawn) did, however, include additional data potentially relevant to the aquatic classification. The EFSA pesticide peer review conclusion on the active component azadiractin (EFSA Journal 2010;8(10):1858) also references additional aquatic toxicity data not included in this 2017 CLH report. These include chronic studies, including a 28-day fish NOEC of 0.0047 mg azadirachtin A/L, a

21-day NOEC for Daphnia of 0.27 mg azadirachtin A/L and a 28-day NOEC for Chironomus of 0.0016 mg azadirachtin A/L, all based on a study on technical azadirachtin. Other endpoints are derived based on the azadirachtin composition of the whole extracts tested.

Comments received on the previous 2014 CLH reports on azadirachtin and the PT 18 Margosa extracts did question the use of chironomid data and how they were derived. However, we consider that chironomid data could be relevant for classification where measured (either initial, mean or time-weighted - to be decided) concentrations in the water phase can be determined. Whilst, as discussed in this present report, the studies on other whole extracts of Margosa might not be entirely relevant to this CO2 extract, this 'substance' and its individual active components do have insecticidal properties, we therefore feel use of these additional data should not be ruled out. Presumably the other limnoids will also have similar toxicity to azadirachtin and their total contribution to the overall chronic toxicity to fish and invertebrates could be assumed. Although their low total percentage will be relevant - given the uncertainties mentioned above regarding the composition and degradability of the individual active components, we feel that further consideration could be given to their likely contribution to the overall extract chronic toxicity. This could perhaps be done by using one of the mixture classification calculations?

Dossier Submitter's Response

1. As you explain we agree that the given information regarding the identity in the dossier seems to be inconsistent. However the composition of an UVCB substance is often confidential. Therefore the result of the 5 batch is stated in the confidential Annex.

2. We fully agree with UK. However, the different time lines of the CLH process and the substance approval within the BPR have to be kept in mind. In this context it should be noted that the CLH proposal has been submitted to ECHA in December 2015, before the substance discussion at BPC WG ENV took place in 2016,where the PBT status of margosa extract was finally reconsidered as being potentially P in December 2016 (based on the not rapidly degradability of the limonoid azadirachtin as the ecotoxicologically relevant component in the UVCB substance margosa extract). Therefore the CLH proposal currently under discussion cannot and does not reflect the WG decision regarding the persistence for the environmental risk assessment (potentially P based on the limonoids). In contrast to our initial assessment, it is now necessary to take the decision at the BPC WG ENV into account and accordingly the UVCB substance margosa extract has to be considered as not rapidly degradable.
According to the Guidance on the Application of the CLP Criteria (Version 4.0 – November 2013) an UVCB substance should be regarded as not rapidly degradable if it contains not-rapidly-degradable constituents with a proportion of ≥20 % or in case the constituent is hazardous, of even lower proportions. It has to be noted that there is actually no lower limit given in the guidance, but we propose to apply the CLP cut off-values to trigger the consideration for classification. In case of the UVCB substance margosa extract, this applies to the components azadirachtin, nimbin, and salanin. Azadirachtin (as the constituent possessing the best data basis of all three limonoids) is classified as not readily biodegradable based on an OECD 301 F test (21.6% mineralisation in 28 days), a study that has been submitted to DE within the biocidal approval process of "margosa extract" for the use as insecticide in PT 18 (CLH-dossier resubmitted to ECHA as " margosa extract from the kernels of Azadirachta indica extracted with water and further processed with organic solvents). For all limonoids, hydrolysis half-lifes in the pH range 4-

9 exceed the trigger of 16 days, whereas no information is available on the identity of the hydrolysis products.

3. With regard to the use of chronic algae data it should be noted that the NOE_rC = 1.05 mg/L slightly exceed the trigger for chronic classification (category 3 if NOEC <= 1 mg/L) and therefore was not considered. As we now consider margosa extract as not rapidly degradable (see above), indeed a classification in category chronic 3 would result, as LC_{50} for fish fulfils the relevant criterion (> 10 to ≤ 100 mg/L, applying the surrogate system according to Guidance on the Application of the CLP Criteria, table 4.1.0 (b)(iii)).

With regard to the use of data from the EFSA pesticide peer review, EFSA Journal 2011;9(3):1858, these data were originally included in a separate CLH-dossier for the PPP a.s. azadirachtin. However, this and a second CLH-dossier for the BP a.s. "margosa extract" (approved for the biocidal use as insecticide PT 18) were withdrawn in 2015 after the substance identity had to be redefined based on the ECHA "Guidance for identification and naming of substances under REACH and CLP" and the guidance "Botanical Active Substances Used in PPP". Currently four different "margosa substances" are formally identified based on the origin of the plant material in combination with the extraction / manufacturing method. The CLH-proposal at hand is for "margosa extract, cold-pressed oil of Azadirachta indica seeds without shells extracted with super-critical carbon dioxide", which is approved for the biocidal use as insect repellent (PT19) and consequently we only included test data for exact this margosa extract (cold-pressed CO2 extract from seeds without shell).

Also from an ecotoxicological point of view these extracts, the cold-pressed CO2 extract from seeds without shell (PT 19) and extract from the kernels extracted with water and further processed with organic solvents (PT 18) should not be considered as equivalent, because there is a fundamental difference concerning the content of the ecotoxicological relevant components azadirachtin A and B, namely < 0,2% in total for the repellent (PT 19) versus 34% for azadirachtin A for the insecticide (PT 18). With regard to the other limonoids, salannin and nimbin they are exceeding the concentration of azadirachtin for this margosa extract (cold-pressed CO2 extract from seeds without shell, PT 19), whereas for the extracts with mainly insecticidal mode of action these limonoids are only minor constituents. This is why we do not consider the data for the other different margosa extracts (e.g. PT 18 insecticide) to be relevant for this margosa extract at hand (repellent PT 19).

We are not aware of any ecotoxicologial effect studies performed with only azadirachtin A. The studies mentioned by UK from EFSA Journal 2011;9(3):1858 have been performed with a test substance called "azadirachtin technical", defined as: "Azadirachtin technical is an extract from seed kernels of the tropical neem tree *Azadirachta indica*. Azadirachtin A is regarded as lead substance." This extract contains even less azadirachtin than the other margosa extracts assessed within BPR or PPP regulation. We have not found a statement in the EFSA pesticide peer review conclusion that "Azadirachtin technical" was purified with regard to azadirachtin A, only the study results were additionally recalculated based on the content of the lead substance azadirachtin A.

Furthermore, the content of azadirachtin A itself in the margosa extract CO2 itself is below the cut off-value to be taken into account for classification (<0.1 % w/w) and therefore would not justify further consideration of respective ecotoxicologial effect studies for classification. A read-across approach from Azadirachtin A to the other

limonoids contained was not considered as appropriate as discussed below and sufficient effect data for the whole extract are available to derive an appropriate classification with regard to the environment. The situation for rapid degradation is a different one, as read-across for rapid degradation from azadirachtin to nimbin and salannin was considered as appropriate. Based on the current data situation, we therefore not recommend to include effect studies performed with other extracts from neem tree in this CLH report.

With regard to the potential ecotoxicological contribution of the other limonoids present in the margosa extract CO2 we would not recommend to apply read-across assuming similar toxicity. It was concluded in the revised assessment report under BPR that the other limonoids show similar modes of action but they seem to be less toxic (e.g. based on *in vitro* assays), but a quantification of biological effect strength was not possible. Therefore lower toxicity was assumed, or as a worst-case at the maximum a similar toxicity. We do not consider a classification on a worst-case assumption (that nimbin, salannin, and also azadirachtin B, are equal toxic as azadirachtin A) as justified. Furthermore, scientific literature does not differentiate between azadirachtin A and B. In the updated CAR we now have included further information regarding the effects of the different limonoids as described in scientific literature.

RAC's response

1. RAC agrees that further clarity could be given regarding the expected composition and % w/w ranges of individual key active components in the substance, including the limnoids azadirachtin A and B, nimbin and salannin in the CLH report.

2. RAC agrees with UK comment. As known, degradation studies with complex substances like UVCB substances present problems of interpretation where each constituent of these substances may behave differently, so a more detailed assessment of the degradability of the individual constituents would be required. The ready biodegradility study performed with Margosa extract does not allow to draw conclusion if and to which extent the constituents, in particular the key active ones including the limnoids azadirachtin A and B, nimbin and salannin, degrade. According to the Guidance on the Application of the CLP Criteria (Version 5.0 – July 2017) *an UVCB substance should be regarded as not rapidly degradable if the constituents that are not-rapidly-degradable constitute a significant part of the substance, e.g. more than 20 %, or for a hazardous constituent an even lower content*.
Since no lower limit is given in the guidance, RAC supports the DS's proposal consisting in applying the CLP cut off-values to trigger the consideration for classification.
Taking into account the DS response, azadirachtin is classified as not readily biodegradable based on an OECD 301 F test, a study that has been submitted within the biocidal approval process of "margosa extract" for the use as insecticide in PT 18. It would be worth including this study in the margosa extract CO2 CLH report. For the other limonoids only information about hydrolysis half-lifes are available indicating that in the pH range 4-9 they are above the trigger of 16 days.
Moreover, as reported by the DS, the BPC WG ENV considered the margosa exctract CO2 as potential P on the basis of not ready biodegradability of azadirachtin and lack of information for the other limonoids.
Taking into account these additional information, RAC supports the DS proposal to consider margosa extract as not rapidly degradable.

3. RAC agrees that a surrogate calculation using LC_{50} value of the most acutely sensitive organisms, i.e. fish, should be also considered.

Based on the above new reasoning on degradability of Margosa extract, it should be considered as not rapidily degradable, therefore a chronic 3 classification would result, because LC_{50} =11.5 mg/L for fish fulfils the relevant criterion (> 10 to ≤ 100 mg/L, applying the surrogate system according to Guidance on the Application of the CLP Criteria, table 4.1.0 (b)(iii)). Therefore RAC agrees with the DS conclusion in the comment response. Regarding the use of data from the EFSA pesticide peer review, EFSA Journal 2011;9(3):1858, RAC is aware of the previous history on the substance identity issue, triggering to the withdrawal of two CLH-dossiers for the PPP a.s. azadirachtin and for the BP a.s. "margosa extract" (approved for the biocidal use as insecticide PT 18). As well clarified by DS, currently four different "margosa substances" are formally identified based on the origin of the plant material in combination with the extraction / manufacturing method. RAC agrees with DS response to include only test data for the margosa extract (cold-pressed CO2 extract from seeds without shell) for which a CLH is proposed. Indeed the cold-pressed CO2 extract from seeds without shell (PT 19) and extract from the kernels extracted with water and further processed with organic solvents (PT 18), cited by UK, should not be considered as equivalent, either for the different content of the ecotoxicological relevant components azadirachtin A and B, and for the conclusion of revised assessment report under BPR to not accept a read-across between the two BPs. The main issue is that in both cases the ecotoxicological effect studies are not performed with the relevant active constituent a.s. Azadiractin A, but with UVCB extracts from neem tree with wide variable compositions. For this reason is not considered appropriate to include effect studies performed with other extracts in the current CLH report.