COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

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Substance name: Azadirachtin
CAS number: 11141-17-6
EC number: -

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2014	Germany	GAB Consulting GmbH (on behalf of the three notifiers of the EU Review of Azadirachtin)	BehalfOfAnOrganisation	1

Comment received

The identity of the substance under evaluation does not seem clearly reported:
The presented data correspond to the data submitted for the PPP EU Review of the active substance "Azadirachtin" which was approved according to Regulation 1107/2009 (see Regulation 540/2011). This active substance "Azadirachtin" was defined as a plant extract derived from seed kernels of the tropical neem tree Azadirachta indica. It contains several compounds of the chemical class of limonoids and other naturally occurring plant components. "Azadirachtin A" is a major biologically active component in "Azadirachtin" and was therefore taken as an analytical reference for quantification purposes. This approach is consistent with the recommendations of the Guidance Document on Botanical Active Substances Used in Plant Protection Products (SANCO/11470/2012-rev.8, 20 March 2014).
In contrast to this, the substance information given on the title page and under Part A 1.1 (page 6) mixes the IUPAC name and CAS number of "Azadirachtin A" with the words "Azadirachtin" and "Neem seed extract". The information under Part B 1.1 (page 10) refers only to Azadirachtin A.
It is therefore not clear whether this CLH report and the proposed classification and labelling refer to the extract or to Azadirachtin A.
If this CLH report refers to "Azadirachtin A", please delete "Azadirachtin" and "Neem seeds extract", since these imply that the classification refers to the whole extract. Conversely, if it refers to the extract, the substance name should be adapted by deleting the IUPAC name for Azadirachtin A. Furthermore the M-factors for aquatic toxicity would have to be amended.

The purity in Part A 1.1 (page 6) is given as ≤ 50% (which refers to Azadrachtin A), while the EU review for plant protection products gave the purity of the extracts under review as ≥ 111 g/kg Azadirachtin A.
Please note that 4 entries for the content of constituents are given in Table 6 (Part B 1.2, page 11). The last entry refers to a source of the notifier IAB, for which however (as stated under Part B Point 4 (page 14)) no toxicological data is available and which therefore is not covered by this CLH dossier.
If this CLH report is considered to refer to the classification and labelling of the extract "Azadirachtin", the identity of the substance should be specified so that it does not include the IAB extract.

Please also note that all studies performed on aquatic toxicity were done with "Azadirachtin" (i.e. the whole extract). In order to perform the risk assessments for the PPP EU Review, study endpoints were recalculated to Azadirachtin A assuming as a worst-case that the toxicity was due only to the content of Azadirachtin A in the extract. The endpoints in terms of "Azadirachtin" (i.e. the extract) are much higher.

Date	Country	Organisation	Type of Organisation	Comment number
02.12.2014	Germany	Trifolio-M GmbH	BehalfOfAnOrganisation	2

Comment received

Subject: Azadirachtin has to be regarded as an UVCB substance, referring to Section 1 "Identity of the substance"

Azadirachtin represents a biological extract with a large variability of components and cannot be described by a single molecular formula. Therefore, it has to be evaluated as an UVCB (substance of unknown or variable composition) substance. That means, it has "…to be identified by considering the origin material of the substance and the most relevant steps during the manufacturing process" (cited from ECHA guidance document "Identification and naming of substances under REACH and CLP"). Furthermore, the guidance document also declares: "The consequence of defining a substance as UVCB is that any significant change of source or process would be likely to lead to a different substance."
That is definitely applicable to the active substance Azadirachtin, which is simply the common name for different neem-extracts, available on the market. This different extracts (with Azadirachtin A as the lead substance) have been gathered in a taskforce to simplify the approval process. Although the equality of the three extracts has already been proven concerning the similarity of the composition, it has to be acknowledged that they arise from different extraction procedures. However, on page 10/11 of the CLH report the active ingredient Azadirachtin is identified by the chemical name, molecular formula and the CAS-Nr. of the lead substance Azadirachtin A.
In contrast to this, the CLH report of the biocidal active substance Margosa ext., which is identical to the a.i. NeemAzal®technical (both of the applicant Trifolio-M) regards it as a UVCB-substance and does not identify the active ingredient by the chemical name, and molecular formula of the lead substance Azadirachtin A. on p. 10 of the CLH report for Margoas ext., the manufacturing process is described in the place for the IUPAC name. That leads to the strange situation that the same substance (obtained from the same extraction procedure and with identical composition) is divided in two different substance-groups during the CLH-procedure.

Subject: Differentiation of the classification regarding toxicity properties of the extracts – general comment referring to Sections 4 (Human health hazard) & 5 (Environmental hazard)

In the CLH-report it is mentioned (p. 14) that the chemical compositions of the three extracts evaluated under the PPP procedure are distinct. Although it further states (p. 15) that there was a conclusion on a toxicological equivalence of the extracts, this is true only for some toxicity properties, as it is shown in Appendix A (list of endpoints for the active substance and the representative formulations) of the "Conclusion on the peer review of the pesticide risk assessment of the active substance azadirachtin" (EFSA Journal, 2011; 9(3):1858), which demonstrates that the single Neem seed kernel extracts (NeemAzal technical, Fortune AzA technical and NPI-720), do have different endpoints in several toxicological categories, especially regarding the risk to aquatic organisms.
Therefore, Trifolio-M GmbH would recommend to follow the proposal of the European Food Safety Authority (EFSA), to differentiate the classification of the three extracts, as EFSA did

in the "Conclusion on the peer review of the pesticide risk assessment of the active substance azadirachtin" (EFSA Journal, 2011; 9(3):1858), with respect to these properties, instead of taking the worst endpoint for all extracts (e.g. p. 61 of CLH-report)
The EU already approved this suggestion for classification in 2011: In section 5 of the "Review report for the active substance azadirachtin" (SANCO/10311/2011 final) it was acknowledged that "the most important endpoints were identified during the re-evaluation process" and "are listed in the EFSA conclusion".

Date	Country	Organisation	Type of Organisation	Comment number
05.12.2014	France		MemberState	3

Comment received

MS-FR agrees with the classification proposed for Human Health Hazards. We are wondering why Azadirachtin is not classified as Repr.2 H361f. Please argue it.
We agree with the classification and M factors proposed for Environmental hazards.

We have a specific comment regarding Part A 1.1 Table 1 (P.6): based on the composition of each constituent of the substance (table 6), the degree of purity should be expressed as a range for better clarity.

Date	Country	Organisation	Type of Organisation	Comment number
05.12.2014	Italy	Federchimica/Agrofarma	BehalfOfAnOrganisation	4

Comment received

Azadirachtin is an extract from the kernels of the Neem tree, Azadirachta indica.
Azadirachtin was included into Annex I of Directive 91/414/EEC by Commission Directive 2011/44/EU (13 April 2011) for use as insecticidal pesticide in the EU. Following entry into force of Regulation (EC) No 1107/2009, Azadirachtin is now included in the Annex to Commission Implementing Regulation (EU) No 540/2011.
At the end of the EU evaluation process of Azadirachtin under Dir. 91/414/EEC, EFSA proposed the following classification with regard to toxicological data: R43 (Skin Sens. 1, H317); no classification for teratogenic potential was stated, even though during the review the EU Rapporteur Member State (DE) had proposed the R63 (Repr. 2, H361d) risk phrase. In the CLH Report recently prepared by Germany for the Harmonized Classification and Labelling of Azadirachtin, the proposal for R63 risk phrase was reiterated.

Basing on the existing data, on our opinion, the conclusion may be different as reported in a recent paper appeared in the literature (1). Summarizing, the overall toxicological database of Azadirachtin showed low incidences of malformations, all within the historical control data. In one teratology study the presence of small interventricular septal defects (an anomaly) was observed at the top two doses and seen only in the presence of marked maternal toxicity. This was considered a secondary non-specific consequence of the maternal toxicity and not a direct developmental effect. Moreover, no effects on the developing heart were reported in a teratogenicity study in rabbits and no indication of adverse developmental effects were noted in a 2-generation study. There are no findings in the rat developmental toxicity studies that would warrant classification for developmental toxicity.

In the light of these observations Federchimica/Agrofarma believe that taking into account all data now available on this plant extract it is appropriate to revise the classification of Azadirachtin with regard to reproductive toxicity.

(1) Srivastava MK, Raizada RB. (2007). Lack of toxic effect of technical azadirachtin during postnatal development of rats. Food Chem. Toxicol. 45(3): 465-71.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2014	Germany	GAB Consulting GmbH (on behalf of the three notifiers of the EU Review of Azadirachtin)	BehalfOfAnOrganisation	5

Comment received

In the CLH report, a classification as developmental toxicant (Repr. 2; H361d) was proposed mainly based on the finding of visceral malformations, namely the incidences of ventricular septal defects (VSD), in a teratogenicity study in the rat. However, these incidences of VSD are rather common observations in rats and were very close to historical control data for the laboratory and well within the published historical control data of MARTA (1996). The treatment also caused maternal toxicity in the dams, at both the mid-dose and high-dose group, noted as reduced body weight gain. Although this effect was only transient, it occurred around days 6 to 8 of pregnancy, which has been identified as critical time in the development of the foetal heart. It is, therefore, reasonable to assume that the observed low incidence of VSD is a high dose effect secondary to maternal toxicity rather than a direct effect of Azadirachtin.
Furthermore, no indications of adverse effects on the developing heart were noted in a 2-generation study, in another teratogenicity study in rats, in a teratogenicity study in rabbits, in several supplemental reports and published data on developmental toxicity studies on Azadirachtin. Based on the experimental evidence it is appropriate not to classify Azadirachtin with regard to reproductive toxicity. Please refer to the additional statement Pfau (2014): Azadirachtin: Evaluation of Classification and Labelling Proposal with regard to Developmental Toxicity, report no. 234379-A2-050601-01.

Date	Country	Organisation	Type of Organisation	Comment number
02.12.2014	Germany	Trifolio-M GmbH	BehalfOfAnOrganisation	6

Comment received

Subject: Setting of specific concentration limits for toxicity for reproduction, referring to Section 4.10 of the CLH report

We, Trifolio-M GmbH are to all intents and purposes convinced that a classification with Repr.2; H361d is not appropriate.

Independently of our judgement, we acknowledge that the Authorities might come to another conclusion. However, in the case of classification with Repr. 2; H361d, we think specific concentration limits (SCL) above the generic concentration limits (GCL) regarding reproduction toxicity have to be set for Azadirachtin via the CLH procedure. We want to refer on the recommendation noted in the Guidance on the Application of the CLP Criteria (2013): "According to CLP article 10, (…). SCLs above the GCL may be set where adequate and reliable scientific information shows that the hazard of a substance is not evident at a concentration above the GCLs. Normally substances that fulfil the criteria for reproductive toxicity are subject to a harmonised classification an labelling and included in Annex VI to CLP. In such cases, SCLs are set via the procedure for harmonisation of classification and

labelling of substances in line with CLP Article 37."(1)
The setting of SCLs is based on the determination of an ED10. According to the guidance document "for effects that are measured as changes in incidence, such as an increase in the number of malformations or resorptions, the ED10 is defined as the dose level at which 10% of the test population above the incidence in the concurrent control shows the effect."(2)
When taking the data of the relevant study (Myers & Dawe, 1997; CLH-report p.41-42) into account, Azadirachtin has to be regarded as a borderline case with low incidences of critical observations and a high dosage of the test substance (up to 1000 mg/kg bw/day). In this study, even the values of the highest dose group did not exceed the ED10 level, neither concerning percentage malformation per foetuses, nor percentage per litters. Theoretically an ED10 >1000 mg/kg bw/day would have been calculated.
Substances which are classified in Category 2 for reproductive toxicity with an ED10 >400 mg/kg bw/day can be placed in potency group 3 (low potency) leading to SCLs above 3%.

Therefore, in the case that Repr. 2 H361d will be committed in the final conclusion of the CLH procedure, we claim to associate Azadirachtin with the low potency group 3, referring to an ED10 ≥400 mg/kg bw/day.
This would result in the setting of the SCL between 3% and 10%, leading to an appropriate classification of the related products.
According to the Guidance on the Application on the CLP Criteria, "the limit of 10% may be considered in certain cases, such as for substances with an ED10 value above 1000 mg/kg bw/day and a NOAEL below 1000 mg/kg bw/day."(3)

Footnotes:
(1) Guidance on the Application of the CLP Criteria, Version 4.0 – November 2013, Annex VI: Background Document to the Guidance for Setting Specific Concentration Limits for Substances Classified for Reproductive Toxicity According to Regulation (EC) No 1272/2008, p. 646
(2) Guidance on the Application of the CLP Criteria, Version 4.0 – November 2013, p. 424
(3) Guidance on the Application of the CLP Criteria, Version 4.0 – November 2013, p. 430

Date	Country	Organisation	Type of Organisation	Comment number
05.12.2014	France		MemberState	7
Comment received				
P24: part 4.7.1.1 P43: part 4.10.2.2 Please argue the non classification of Azadirachtin as Repr.2 H361f, considering findings with respect to fertility described for humans in open literature (spermicidal effects in vitro, intravaginal/-uterine used contraceptive) and effects on male and female sexual organs observed in subacute and subchronic toxicity studies in rats (changes in ovary weight, decreased number of corpora lutea, endometrial atrophy in uterus, marked atrophy in testes seminiferous tubular), taking into account that no further mechanistic studies have been submitted to definitively rule out an effect of the active substance on the reproductive system.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2014	Germany	GAB Consulting	BehalfOfAnOrganisation	8

		GmbH (on behalf of the three notifiers of the EU Review of Azadirachtin)		

Comment received

In the CLH report, a classification as Skin Sensitising Category 1 (without sub categories) is proposed since study results for two tested extracts led to the classification 1B, while one study led to classification 1A.
It is proposed that classification as 1B is sufficient given that sensitisation studies with the formulated products did not show any sensitising effects. Please refer to the additional statement Pfau (2014): Azadirachtin: Evaluation of Classification and Labelling Proposal with regard to Skin Sensitisation, report no. 234379-A2-050206-01.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
04.12.2014	Germany	GAB Consulting GmbH (on behalf of the three notifiers of the EU Review of Azadirachtin)	BehalfOfAnOrganisation	9

Comment received

Please note that all studies performed on aquatic toxicity were done with "Azadirachtin" (i.e. the whole extract). In order to perform the risk assessments for the PPP EU Review, study endpoints were recalculated to "Azadirachtin A" assuming as a worst-case that the toxicity was due only to the content of "Azadirachtin A" in the extract. The endpoints in terms of "Azadirachtin" (i.e. the extract) are thus much higher.
The endpoints given in Table 44 (Part B Point 5.4 on page 60) and Table 45 (Part B Point 5.4.1.1 on page 60), and also the value of 0.048 mg/L mentioned directly below that table refer to "Azadirachtin A". In the same way, endpoint values in Table 47 (Part B Point 5.4.1.2 on page 62), Table 49 (Part B Point 5.4.2.1 on page 65), Table 51 (Part B Point 5.4.2.2 on page 67), Table 53 (Part B Point 5.4.3 on page 69), and Table 55 (Part B Point 5.4.4 on page 71) are recalculated values for "Azadirachtin A".

The M factors and SCL (according to DSD) calculated in Part B, Point 5.5 (page 75) of the CLH report were derived for Azadirachtin A (after recalculating the results for the extracts to the content of pure Azadirachtin A).
In case the substance under review in this CLH report is "Azadirachtin" (i.e. the extract), the values presented under that point should read:
The lowest acute endpoint for Azadirachtin technical is the LC50 of 0.48 mg Azadirachtin technical/L.
The lowest chronic endpoint for Azadirachtin technical is the NOEC of 0.01 mg Azadirachtin technical/L.

Classification according to the CLP:
The acute M-factor is 1 instead of 10.

Classification according to the DSD:
Based on the LC50 of 0.48 mg Azadirachtin technical/L
Azadirachtin technical fulfils criteria for classification with N; R50-53 with an SCL of:
Cn ≥ 25%, N; R50-53
2.5% ≤ Cn < 25%, N; R51-53
0.25% ≤ Cn < 2.5%, R52-53

Please also note that the lowest NOEL of 0.01 mg/L was derived for Chironomus riparius, which is neither a fish, nor a crustacea, nor an algae, and thus does not belong to the indicator species proposed by Regulation (EC) No 1272 /2008 for chronic toxicity classification.

Degradation in water:
In the CLH report it was concluded from standard screening tests that Azadirachtin variants NeemAzal and Neem Seed Extract as well as the analytical leading compound Azadirachtin A are not readily biodegradable. It was also found in these studies that all substances are not inhibitory.
Based on further evidence presented below, it is shown that the analytical leading compound Azadirachtin A exhibits rapid degradability. It is expected that the other components of Azadirachtin will show similar behaviour. Hence, the classification and labelling of Azadirachtin have to be reconsidered.

It is true that Regulation (EC) No 1272/2008 mentions biodegradation screening tests (see Point 4.1.2.9.2. of Annex I) as "one way of demonstrating rapid degradation", but also states that "a fail in the screening test does not necessarily mean that the substance will not degrade". It "allows the use of data to show that the substance did actually degrade biotically or abiotically in the aquatic environment by > 70 % in 28 days. Thus, if degradation is demonstrated under environmentally realistic conditions, then the criterion of 'rapid degradability' is met." However, under Point 4.1.2.9.3. of Annex I it is also stated that "primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment."

As shown in the CLH report Azadirachtin A in water is mainly subject to base-catalysed hydrolysis. Also indirect photolysis and microbial degradation are expected to contribute significantly to the degradation of Azadirachtin A in natural water bodies under conditions of use.
As also presented in the CLH report a rapid disappearance of Azadirachtin A was confirmed in the water metabolism study by Molinari (2002), resulting in a DT50 value in water of 13.7 days (20 °C), clearly below the trigger value of 16 days. Since a follow-up of degradation products is not possible because radio-labelling and substance synthesis are not feasible (see also Point 5.1.2.3 of CLH report), information on degradation products could not be derived from this study. First evidence is available from the biodegradation screening tests, confirming no relevant inhibition of microbial activity in STP effluent.
Detailed information is available from the following studies, all part of the Annex I inclusion procedure of Azadirachtin:
In order to characterise the degradation products of Azadirachtin with regard to their ecotoxicological potential, fish and aquatic invertebrates were exposed to aged NeemAzal residues in water (Teigeler, 2009; Simon, 2009).
NeemAzal was applied into the water phases of two water-sediment systems of different trophic conditions to give a final concentration of 45 mg NeemAzal/L, corresponding to 13.8 mg Azadirachtin A/L, a concentration at which effects on fish and aquatic invertebrates could be expected. Water samples were taken 1 hour, and on day 3, day 7, day 14 and day 21 after treatment for chemical analysis (Geschke, 2009) and for the exposure of test animals.
In the subsequent bioassays fish (Oncorhynchus mykiss) and water fleas (Daphnia magna) were exposed to these (undiluted or diluted) water samples to determine the effects of NeemAzal water residues.
In the study on fish, neither in the first bioassay with Oncorhynchus mykiss performed one

hour after application of NeemAzal to the water-sediment system, nor in consecutive bioassays with water samples containing degradation products of NeemAzal, effects on fish were observed up to the highest test rate.
In the study on Daphnia magna, a clear dilution-response relation was observed for both water sources in the first two bioassays performed with water samples taken 1 hour or 3 days after application, resp. The EC50 values were determined to be 11.3 and 11.4 mg initial NeemAzal/L for 1-hour water samples of the clayey silt water-sediment system and sandy water-sediment, respectively. For 3-days water samples the EC50 values were determined to be 28.7 and 31.7 mg initial NeemAzal/L for the clayey silt and sandy water-sediment systems, respectively. In the following bioassays the EC50 values were > 45 mg initial NeemAzal/L for aged water samples from both sediment-water systems.
The decline of effects was thus correlated to the decline of Azadirachtin A in the water samples confirming that degradation products of Azadirachtin in water are significantly less toxic than the unaltered Azadirachtin.
Further details can be found in the statement by Otto & Häusler (2009)): Statement on the relevance of degradation products of Azadirachtin in aquatic systems, report no. 234379-A3-0708-02, attached to this submission.

Hence, the criteria for rapid degradability – fast removal from the environment and non-hazardousness of degradation products – are met by Azadirachtin A.
Further evidence is based on the bioaccumulation potential and LogPow of Azadirachtin A which are significantly below the trigger value as fixed in Regulation (EC) No 1272/2008.

Date	Country	Organisation	Type of Organisation	Comment number
03.12.2014	Italy		BehalfOfAnOrganisation	10
Comment received				
It is our opinion that test results on Chironomus riparus should not be used to classify the substance as being a chronic hazard to the aquatic environment. The study results were used to make a risk assessment when the substance was evaluated as an insecticide which is different from being used for hazard classification under Reg, 1272/2008. The classification with respect to chronic hazard to the aquatic environment should be based on study results from fish, crustacae or algae only. CLH report p.75/76				

ATTACHMENTS RECEIVED:

1. **Azadirachtin: Evaluation of Classification and Labelling Proposal with regard to Developmental Toxicity**, report no. 234379-A2-050601-01 (refer to comment 5)
2. **Azadirachtin: Evaluation of Classification and Labelling Proposal with regard to Skin Sensitisation**, report no. 234379-A2-050206-01 (refer to comment 8)
3. **Statement on the relevance of degradation products of Azadirachtin in aquatic systems**, report no. 234379-A3-0708-02 (refer to comment 9)