Statement on the classification of the substance Margosa, ext. in the CLH report with respect to Aquatic Toxicity

Date: 02 December 2014

Author:

Trifolio-M GmbH
Dr.-Hans-Wilhelmi-Weg 1
35633 Lahnau
Deutschland

Phone: +49 6441 20977-0
Fax: +49 6441 20977-50
Email: info@trifolio-m.de

Content

INTRODUCTION 3

INITIAL SITUATION 3

Endpoints of studies with *Chironomus riparius* 3

Consulting studies with the midge *Chironomus riparius* 5

Discussion 6

CLASSIFICATION OF MARGOSA, EXT. PROPOSAL OF TRIFOLIO-M 7

CONCLUSION 8

REFERENCES 8

Introduction

In the "CLH report Proposal for Harmonised Classification and Labelling Substance Name: Margosa, ext." the substance Margosa, ext. is classified as Aquatic Chronic, Category 1, H410 with an M-Factor of 10 regarding the risks to the environment.
Trifolio-M does not share this view with respect to the available studies. At the beginning of this statement, the initial situation is described followed by the argumentation why Trifolio-M does not agree with the appraisal of its active substance. Finally, a counter-proposal is presented.
To avoid misunderstandings in advance, a short definition is given here;

> The active substance Trifolio-M is working with, is a natural plant extract from neem (*Azadirachta indica*) seed kernels. It can be identified and detected by analysis of its lead component Azadirachtin A. "Margosa, ext." is the name of this active substance when biocides are concerned. In the case of plant protection products (NeemAzal®-T/S), the identical active substance is named "NeemAzal® technical".

In the studies, which are cited herein and are used for registration purposes, the active substance name NeemAzal® technical is being used. Thus, when the text refers to NeemAzal® technical this can be read as Margosa, ext., because it is identical.

Initial Situation

At first, the points of departure are compiled, thereafter it will be explained how this situation came to be and finally this process will be discussed. Although Trifolio-M has submitted other studies with respect to an impact on the aquatic environment too, this statement takes account to the studies on *Chironomus riparius* only, since their results stipulated finally the given endpoints concerning the aquatic toxicity

Endpoints of studies with *Chironomus riparius*

Figure 1 shows the endpoints of the two long-term studies with larvae of the midge *Chironomus riparius* (Diptera: Insecta) using the active ingredient NeemAzal® technical (Gonsior, 2008a) and the product NeemAzal®-T/S (Gonsior, 2008b).

Table 1: EC_x- values of chironomids exposed to NeemAzal®-T/S determined with the nominal concentrations

	NeemAzal®-T/S [mg/L]
NOEC	0.573
EC_{50}	1.15
95 % confidence limit of EC_{50}	0.811 – 1.49

Table 1: EC_x- values of chironomids exposed to NeemAzal® determined with the nominal concentrations

	NeemAzal® [mg/L]
NOEC	0.0184
EC_{50}	0.0329
95 % confidence limit of EC_{50}	0.0206 – 0.0451

Table 2: EC_x- values of chironomids exposed to NeemAzal®-T/S determined with the actual concentrations

	NeemAzal®-T/S [mg/L]
NOEC	0.433
EC_{50}	0.840
95 % confidence limit of EC_{50}	0.604 – 1.066

Figure 1: Endpoints in two studies with Chironomus riparius (Gonsior, 2008a, left, and Gonsior, 2008b, right)

Although endpoints result from the studies with *Chironomus riparius,* the classification of Margosa, ext. as "Aquatic Chronic, Category 1, H410 with an M-Factor of 10" however 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) (see Figure 2 and
Figure 3).

Table 49: Long-term toxicity to Chironomid larvae

Method / Guideline	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]		Remarks	Reference
			design	duration	NOEC	LOEC		
OECD 219	*Chironomus riparius*	Emergence, development rate	static	28 days	0.0184 (nominal conc.) 0.0075 (mean measured conc.)	0.0368 (nominal)		Gonsior, G. (2008a) report no. 2007/1356/01-ASCr
OECD 219	*Chironomus riparius*	Emergence, development rate	static	28 days	0.018 (nominal conc.) 0.006 (mean measured conc.)	0.036 (nominal)	Study performed with product NeemAzal-T/S, effect values related to active substance Margosa extract	Gonsior, G. (2008b) report no. 2007/1355/01-ASCr

Figure 2: Endpoints of the CLH report for Margosa ext.

Toxicity data for aquatic species (most sensitive species of each group) (Annex IIA, point 8.2, Annex IIIA, point 10.2)

Species	Time-scale	Endpoint	Toxicity (mg a.s./L) [1]
Fish			
Oncorhynchus mykiss	96-h, semi-static,	Mortality, LC_{50}	4.1 [2]
Oncorhynchus mykiss	28-d, flow-through	Mortality, growth, NOEC / LOEC	1.9 / 4.4 [2]
Invertebrates			
Daphnia magna	48-h, static	Immobility, EC_{50}	9.69
Daphnia magna	21-d, semi-static	Reproduction, NOEC / LOEC	0.1 / 0.22[2]
Chironomus riparius	28 d static	Emergence, development rate NOEC	0.006[2]
Algae			
Scenedesmus subspicatus	72-h, static	Growth rate, E_rC_{50} Biomass, E_bC_{50} Growth rate, E_rC_{10}	1041 482 332
Microorganisms			
Activated sludge predominately from municipal wastewater treatment plant	30 min, static	Respiration inhibition (oxygen consumption), NOEC	1000

[1] a.s. refers to margosa extract; [2] test conducted with NeemAzal-T/S containing 4% margosa extract

Figure 3: Endpoints of the EU for Margosa ext.[1]

The approach of calculating the endpoint was selected due to the content of the submitted studies. The poor recovery rates of the lead component in these studies (below the limit of quantification (LOQ), see
Figure 4), were the reason, why the actual amount measured was not considered to determine the NOEC. Instead, the evaluators calculated mean values by using the resulting endpoint and the LOQ/2 of the study.

Table 12: Amount of NeemAzal® in water, pore water and sediment in mg/vessel during the main test

Time	Sample	Water	Pore water	Sediment	Total/vessel
[d]	NeemAzal® [mg/vessel]	NeemAzal® [mg/vessel]			
0	0	< LOQ	< LOQ	< LOQ	< LOQ
	0.0184	0.0160	0.0017	< LOQ	0.0177
	0.294	0.1015	0.0073	< LOQ	0.1088
7	0	< LOQ	< LOQ	< LOQ	< LOQ
	0.0184	< LOQ	< LOQ	< LOQ	< LOQ
	0.294	< LOQ	< LOQ	< LOQ	< LOQ
28	0	< LOQ	< LOQ	< LOQ	< LOQ
	0.0184	< LOQ	< LOQ	< LOQ	< LOQ
	0.294	< LOQ	< LOQ	< LOQ	< LOQ

LOQ = 0.00625 mg/L for water and pore water; 0.0156 mg/kg for sediment

Table 13: Amount of NeemAzal®-T/S in water, pore water and sediment in mg/vessel during the main test

Time	Sample	Water	Pore water	Sediment	Total/vessel
[d]	NeemAzal®-T/S [mg/vessel]	NeemAzal®-T/S [mg/vessel]			
0	0	< LOQ	< LOQ	< LOQ.	< LOQ
	0.573	0.4326	< LOQ	< LOQ	0.4326
	9.17	3.2930	0.2516	< LOQ	3.5446
7	0	< LOQ	< LOQ	< LOQ	< LOQ
	0.573	< LOQ	< LOQ	< LOQ	< LOQ
	9.17	< LOQ	< LOQ	< LOQ	< LOQ
28	0	< LOQ	< LOQ	< LOQ	< LOQ
	0.573	< LOQ	< LOQ	< LOQ	< LOQ
	9.17	< LOQ	< LOQ	< LOQ	< LOQ

LOQ = 0.183 mg/L for water and pore water; 0.475 mg/kg for sediment

Figure 4: Analytical data of the study Gonsior 2008a with the active ingredient (left) and Gonsior 2008b with the product (right)

Consulting studies with the midge *Chironomus riparius*

The classification of Aquatic Chronic, Category 1, H410 in the CLH Report for the substance Margosa, ext. has been triggered by studies with *Chironomus riparius.*

[1] Directive 98/8/EC concerning the placing biocidal products on the market (Inclusion of active substances in Annex I or IA to Directive 98/8/EC)- Assessment Report Margosa Extract Product-type 18

In the CLH Report it is stated on page 65: *"Although this is not a standard test system for classification, the use of this value is justified by the insecticidal mode of action of the substance as well as by the fact that exposure of the test organisms was predominantly via the water face"*.
The respective passages in the CLP-regulation (EC) No. 1272/2008 amended by regulation (EU) No. 286/2011 (Annex I 4.1.2.7)[2] concerning tests of other species than fish, crustacae and algae reveal that only in the classification of acute toxicity other species than fish, crustacae or algae can be used for the classification. When chronic toxicity is concerned the NOECs or other equivalent ECx (e.g. EC_{10}), generated according to the standardized test methods, i.e. studies with fish, crustacae and algae, shall be used for classification.

4.1.2.7. *Aquatic toxicity*

4.1.2.7.1. Acute aquatic toxicity is normally determined using a fish 96-hour LC_{50}, a crustacea species 48-hour EC_{50} and/or an algal species 72- or 96-hour EC_{50}. These species cover a range of trophic levels and taxa and are considered as surrogate for all aquatic organisms. Data on other species (e.g. Lemna spp.) shall also be considered if the test methodology is suitable. The aquatic plant growth inhibition tests are normally considered as chronic tests but the EC_{50}s are treated as acute values for classification purposes (see note 2).

4.1.2.7.2. For determining chronic aquatic toxicity for classification purposes data generated according to the standardised test methods referred to in Article 8(3) shall be accepted, as well as results obtained from other validated and internationally accepted test methods. The NOECs or other equivalent EC_x (e.g. EC_{10}) shall be used.

Figure 5: Passage from regulation (EU) No. 286/2011

Discussion

Trifolio-M comprehends how the initial situation arises (as illustrated), but does not agree with that approach for the following reasons:

1. *Chironomus riparius* should not be considered for long-term (chronic) aquatic toxicity-classification. Trifolio-M does not want to harm this type of species, represented by *Chironomus* at all, however, in order to protect these species, other measures, like buffer zones to natural waters, have been implemented in the product registration by the authorities. These measures were based on aquatic toxicity values that were found to be most critical for *Chironomus riparius*. With these buffer zones <u>a harmful effect on the midge can be excluded</u>.
2. In the light of the mode of action of Margosa, ext. the long-term studies with *Chironomus riparius* seem to be irrelevant; From efficacy trials with the plant protection product, it is known that NeemAzal® technical has a slow mode of action to several insects. It does not kill the insects immediately, but influences their development. After exposure to NeemAzal® technical insects usually stop feeding. Furthermore, moulting and egg fertility

[2] OJ L 83, 30.03.2011, p. 23

are affected. Therefore, no conclusion concerning long-term toxicity of the corresponding studies could be taken since the test organisms could have been already poisoned from the very beginning; even the effects were only shown after a few days. Also the contents of active substance after 7 or 28 days are not crucial since the effects of long-term or acute toxicity would be the same.

3. The results of the analytics of the concerned studies (see
4. Figure 4) are not in accordance with the experiences from other studies:
    - Molinari (2002) determined a DT_{50} value of 13,7d concerning Azadirachtin A in a water sediment system (pH value ~7)
    - Troß (1996) extrapolated a half-live of Azadirachtin A of 4,4 days in a hydrolysis study with an pH value of 8 (this is close to the pH value in the *Chironomus riparius* studies)

    Thus, Trifolio-M had expected a recovery rate between 20% and 75% but definitive not below the LOQ. The consideration of the resulting recovery rates of the Chironomus studies is not realistic.
5. Trifolio-M does not comprehend in general, why a study endpoint, measured in valid studies that were done according to the respective OECD guideline and under GLP is not accepted. The resulting approach to calculate the mean value seems rather arbitrary than scientifically verifiable. In consequence, it leads to the presumption that the higher the LOQ the higher is the NOEC.

    Irrespective of the disagreement of Trifolio-M to the consultation of the *Chironomus riparius* studies for the long-term aquatic toxicity, if these studies are to be considered, the NOEC of 0,0184 mg a.s./l (see Figure 2) should be defined (which also means no M-value).
6. Trifolio-M submitted several other studies with eligible test organisms, which are valid and accordingly convenient to evaluate the behaviour of Margosa, ext. concerning long-term aquatic toxicity.

Classification of Margosa, ext. proposal of Trifolio-M

Followed by the conclusion not to agree with the classification proposed in the CLH report, Trifolio-M performed its own evaluation of the active substance Margosa, ext., according to council regulation (EC) No. 1272/2008 amended by regulation (EU) No. 286/2011.
Trifolio-M has provided valid studies with fish, crustacae and algae – species, which are relevant for classification (see Figure 5) – that enables to categorise the active substance and to establish the needed NOEC values. As shown in
Figure 3 for long-term effects the lowest endpoint of fish, crustacae and algae is the NOEC = 1.84 mg a.s./L from the study with *Daphnia magna (*Schmitz, 2009), which does not trigger a classification.

Conclusion

Conclusively, in view of the information provided in this statement, the company Trifolio-M does not see the need to classify the active substance Margosa, ext. with respect to long-term aquatic toxicity, because it is not proven to harm *Chironomus riparius* in a long-term and the NOEC endpoints measured for fish, crustacae and algae are higher than 1 mg/L.

If the data on chironomids would still be taken into account, the classification should only be Aquatic chronic 2, H411 and no M-factor should be given, due to the EU-agreed endpoint of 0.0184 mg extract/L.

References

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

Molinari G.P. (2002) Azadirachtin behaviour in soil and in water: water metabolism, Report-no. SIPCAM/01/04/Azadirachtin/metacqua, GLP: yes, Published: no

Schmitz A. (2009) Effects of NeemAzal®-T/S on the Reproduction of *Daphnia magna*, Report-no. TRF-002/4-21, GLP: yes, Published: no

Troß R. (1996) Hydrolytic stability of NeemAzal (German original), Report-no. TM 1195.15, GLP: yes, Published: no