

**Statement on the classification of the substance**

**Margosa, ext. [extracted with water]**

**[from the kernels of *Azadirachta indica* extracted with water and further processed with organic solvents] = NeemAzal technical**

**Setting of specific concentration limits (SCLs) with respect to the classification as developmental toxicant (Repr. 2; H361d)**

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## Introduction

The RMS Germany prepared a CLH-Dossier to define an appropriate harmonized classification for *Margosa, ext. [extracted with water]* and proposed classification in Category 2 (H361d) regarding developmental toxicity. The attention is focused on the teratogenicity study in the rat, especially on visceral malformations and anomalies, namely on incidences of small and severe ventricular septal defect -VSD (Myers and Dawe 1997<sup>1</sup>, evaluated in 4.10.2.1 of the CLH-report).

The critical findings (judged as malformations on the heart or the thoracic circulatory system) in the developmental study occurred in the high dose group (1000 mg/kg bw; 3 fetuses in 3 litters, i.e. one fetus in each concerned litter) and mid dose group (225 mg/kg bw; one fetus). In the high dose group (1000 mg/kg bw) distinct maternal effects are evident. That is because OECD TG 414 for prenatal developmental toxicity studies recommends: "The highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering. At least one intermediate dose level should produce minimal observable toxic effects."<sup>2</sup>. Therefore in tests with substances of low toxicity, a very high dose must be applied to produce any toxicity at all. Hence, the highest dose in the development study with *Margosa, ext. [extracted with water]* had been set to 1000 mg/kg bw in order to obtain maternal toxicity. In other studies with substances of severe toxicity, the highest doses are much lower. Even at the mid dose (225 mg/kg bw), slight maternal effects (decreased food consumption, post dosing salivation) are noted.

Taking into account the consequences for the corresponding products, the classification with Repr. 2 seems inappropriate: While the concentration of *Margosa, ext. [extracted with water]* in the formulations is comparably low (range 2,4-4%, on average 3.2%), but being just outside the GCL of 3%, the products ought to be classified with H361d as well, although the effects in the study with the active substance were not significant statistically and occurred at high dosage only. In view of the use patterns of the products, and moreover, the low potential for accumulation in the environment, the relevance of the study outcome for humans (and other vertebrates) can be regarded as low, and a risk as suggested by the classification can be excluded for the products.

In such cases, authorities might assign a special SCL for the corresponding products. Therefore the generic concentration limit (GCL) for *Margosa, ext. [extracted with water]* has to be replaced by a specific concentration limit (SCL), leading to an appropriate classification of the products.

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<sup>1</sup> Myers, D.P., Dawe, I.S. (1997) A study of developmental toxicity in rats (gavage administration) Report-no. 05-293-97, EIP 2/952493,

<sup>2</sup> OECD Guideline for Testing of Chemicals. Prenatal developmental toxicity study. OECD/OCDE 414, 25 June 2018, p. 3

## Justification of potency boundaries and specific concentration limits (SCL) for *Margosa, ext. [extracted with water]* based on ED<sub>10</sub>-calculation

For the setting of specific SCLs, we want to refer to the recommendation noted in the **Guidance on the Application of the CLP Criteria**: “According to CLP article 10, (...). SCLs above the GCL may be set in exceptional circumstances 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 and 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.”<sup>3</sup>

The setting of SCLs is based on the determination of an ED<sub>10</sub> value. According to the guidance document (3.7.2.6.3.2.) “For effects that are measured as changes in incidence, such as an increase in the number of malformations or resorptions, the ED<sub>10</sub> is defined as the dose level at which 10% of the test population above the incidence in the concurrent control shows the effect.”<sup>4</sup>

If the ED<sub>10</sub> exceeds 400 mg/kg bw/day, SCLs above the GCL are warranted. When taking the data of the relevant study (Myers & Dawe, 1997; CLH-report p. 44-48) into account, *Margosa, ext. [extracted with water]* 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 the guidance document (3.7.2.6.3. Determination of the ED<sub>10</sub> value) it is stated: „The ED<sub>10</sub> may be obtained either directly or by linear interpolation from experimental data or estimated using Bench Mark Dose (BMD) software.”<sup>5</sup>

Table 1 presents ED<sub>10</sub> rates and ED<sub>10</sub> values for all incidences of malformations and anomalies, based on the data of Myers & Dawe (1997), performed on a fetuses-based evaluation.

Table 2 presents ED<sub>10</sub> rates and ED<sub>10</sub> values for all incidences of malformations and anomalies, based on the data of Myers & Dawe (1997), and performed on a litter-based evaluation, taking in account the affected proportion of foetuses in each litter calculation, hereby considering the litter effect.

Table 3 presents ED<sub>10</sub> values for the incidences of the treatment-related malformations and anomalies (interventricular septum defect and duplicated inferior vena cava), also performed on a litter-based evaluation considering the litter effect.

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<sup>3</sup> Guidance on the Application of the CLP Criteria, Version 5.0 – July 2017, p. 626, 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

<sup>4</sup> Guidance on the Application of the CLP Criteria, Version 5.0 – July 2017, p. 409

<sup>5</sup> Guidance on the Application of the CLP Criteria, Version 5.0 – July 2017, p. 408

Table 1: ED<sub>10</sub> values based on the study by Myers & Dawe (1997), considering all malformations and all anomalies, performed on a fetus base evaluation

	Control	50 mg/kg bw/day	225 mg/kg bw/day	1000 mg/kg bw/day	ED <sub>10</sub> Rate(%) / ED <sub>10</sub> (mg/kg bw / day)
n fetuses	152	159	152	151	
Visceral malformation [%]	0.00	2.52	0.66	1.99	<b>10,00 / &gt; 1000</b>
n fetuses	152	159	149	149	
Skeletal malformation [%]	0.66	0.63	2.68	3.36	<b>10.66 / &gt; 1000</b>
n fetuses	152	159	152	151	
Visceral anomaly [%]	7,23	11,95	6,58	8,61	<b>17,23 / &gt; 1000</b>
n fetuses	152	159	149	149	
Skeletal anomaly [%]	11,84	5,03	10,07	8,72	<b>21,84 / &gt; 1000</b>

Table 2: ED<sub>10</sub> values based on the study by Myers & Dawe (1997), considering all malformations and all anomalies, evaluated on litter effect basis (considering the proportion of affected fetuses in each litter)

	Control	50 mg/kg bw/day	225 mg/kg bw/day	1000 mg/kg bw/day	ED <sub>10</sub> -Rate (%) / ED <sub>10</sub>
Number of litters examined	23	23	23	23	
Visceral plus skeletal malformation per litter in %	0.27	1.83	1.66	2.38	<b>10.27 / &gt;1000</b>
Visceral anomaly per litter in %	7.19	12.5	6.43	9.63	<b>17.9 / &gt;1000</b>
Skeletal anomaly per litter in %	12.4	5.05	9.68	8.28	<b>22.4 / &gt;1000</b>
Combined (in %, litter based)	19.9	19.4	17.8	20.3	<b>29.9 / &gt;1000</b>

Table 3: ED<sub>10</sub> values based on the study by Myers & Dawe (1997), considering VSD and duplicated inf. Vena cava only, evaluated on litter effect basis (considering the proportion of affected foetuses in each litter)

	Control	50 mg/kg bw/day	225 mg/kg bw/day	1000 mg/kg bw/day	ED <sub>10</sub> -Rate (%) / ED <sub>10</sub>
Number of litters examined	23	23	23	23	
Visceral malformation per litter in %	0	0	0.73	2.47	<b>10 / &gt;1000</b>
Visceral anomaly per litter in %	0	0	1.97	1.24	<b>10 / &gt;1000</b>
Combined in % (litter based)	0	0	2.70	3.71	<b>10 / &gt;1000</b>

Evidently, even at the highest dose group (1000 mg/kg bw/day) the incidences did not exceed the ED<sub>10</sub>-level. Therefore, the ED<sub>10</sub>-dose of *Margosa, ext. [extracted with water]* is supposed to exceed the 400 mg threshold clearly.

Figure 1: SCLs for each potency group and classification category (excerpt from guidance on the Application of the CLP criteria)

	Category 1		Category 2	
	Dose	SCL	Dose	SCL
Group 1 high potency	ED <sub>10</sub> below 4 mg/kg bw/day	0.03% (factors of 10 lower for extremely potent substances <sup>B)</sup> )	ED <sub>10</sub> below 4 mg/kg bw/day	0.3% (factors of 10 lower for extremely potent substances <sup>B)</sup> )
Group 2 medium potency	ED <sub>10</sub> ≥ 4 mg/kg bw/day, and ≤ 400 mg/kg bw/day	0.3% (GCL)	ED <sub>10</sub> ≥ 4 mg/kg bw/day, and ≤ 400 mg/kg bw/day	3% (GCL)
Group 3 low potency	ED <sub>10</sub> above 400 mg/kg bw/day	3%	ED <sub>10</sub> above 400 mg/kg bw/day	3-10% <sup>A</sup>

Substances which are classified Category 2 for reproductive toxicity with an ED<sub>10</sub> above 400 mg/kg bw/day must be placed in Group 3 (low potency) according the guidance

document<sup>6</sup>, leading to SCLs between 3-10% (Figure 1). Hence, in the case that Repr. 2; H361d will be decreed in the final conclusion of the CLH procedure, we claim to associate *Margosa, ext. [extracted with water]* with the low potency group, referring to an ED<sub>10</sub> ≥400 mg/kg bw/day.

## Conclusion

According to the guidance on the application of CLP-criteria “... SCLs are set via the procedure for harmonisation of classification and labelling of substances in line with CLP Article 37.”

Therefore, if *Margosa, ext. [extracted with water]* is judged as H361d in the final decision, a SCL between 3% and 10% must be assigned via harmonised classification in order to ensure an appropriate classification of the related products.

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<sup>6</sup> Guidance on the Application of the CLP Criteria, Version 5.0 – July 2017, p. 415 and 641