Committee for Risk Assessment

RAC

Annex 1
Background document
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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

9 March 2018

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name:

Margosa Extract, 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

Index Number: -

Contact details for dossier submitter:

BAuA
Federal Institute for Occupational Safety and Health
Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
D-44149 Dortmund, Germany

Version number: **2.0**

Date: **December 2016**

CONTENTS

Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name[1]:	Margosa Extract, 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
Annex VI Index number:	
Degree of purity:	100% w/w
Impurities:	None, since the extract is an UVCB substance

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	none
Current proposal for consideration by RAC	none
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	none

[1] *Azadirachta indica*: English - Margosa; Neem Tree

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON MARGOSA EXTRACT, COLD-PRESSED OIL OF *AZADIRACHTA INDICA* SEEDS EXTRACTED WITH SUPER-CRITICAL CARBON DIOXIDE

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				conclusive but not sufficient for classification
2.2.	Flammable gases				
2.3.	Flammable aerosols				
2.4.	Oxidising gases				
2.5.	Gases under pressure				
2.6.	Flammable liquids				conclusive but not sufficient for classification
2.7.	Flammable solids				
2.8.	Self-reactive substances and mixtures				conclusive but not sufficient for classification
2.9.	Pyrophoric liquids				conclusive but not sufficient for classification
2.10.	Pyrophoric solids				
2.11.	Self-heating substances and mixtures				
2.12.	Substances and mixtures which in contact with water emit flammable gases				conclusive but not sufficient for classification
2.13.	Oxidising liquids				conclusive but not sufficient for classification
2.14.	Oxidising solids				
2.15.	Organic peroxides				conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals				conclusive but not sufficient for classification
3.1.	Acute toxicity - oral				conclusive but not sufficient for classification
	Acute toxicity - dermal				conclusive but not sufficient for classification
	Acute toxicity - inhalation				conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation				conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation				conclusive but not sufficient for classification
3.4.	Respiratory sensitisation				data lacking
3.4.	Skin sensitisation				conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity				conclusive but not sufficient for classification
3.6.	Carcinogenicity				data lacking*
3.7.	Reproductive toxicity				conclusive but not sufficient for classification

3.8.	Specific target organ toxicity – single exposure				conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure				conclusive but not sufficient for classification
3.10.	Aspiration hazard				data lacking
4.1.	Hazardous to the aquatic environment				conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer				data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification
* Data lacking is justified in the framework of biocidal active substance approval

Table 4: Proposed labelling based according to the CLP Regulation

	Labelling	**Wording**
Pictograms	none	
Signal Word	none	
Hazard statements	none	
Suppl. Hazard statements	none	
Precautionary statements	none	

Proposed notes assigned to an entry: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

2.2 Short summary of the scientific justification for the CLH proposal

No classification and labelling with regard to the physical hazards are proposed.

Based on the available data no classification for human health hazards is considered necessary.

Based on the available data environmental classification is not required.

2.3 Current harmonised classification and labelling

No entry in Annex VI.

2.4 Current self-classification and labelling

No entry in C&L inventory.

RAC general comment
Different botanical extracts made from *Azadirachta indica* (Synonym: Margosa, Neem) are used as biocidal active substances and are all covered by the same chemical numerical identifiers (EC: 283-644-7, CAS: 84696-25-3). According to the Guidance for identification and naming of substances under the REACH and CLP Regulations (May 2017, Version 2.1), such different extracts should receive different names. Due to different raw materials and extraction methods (e.g. methods using water or other organic solvents), the constituents vary substantially between different extracts. *Margosa CO_2-ext.* therefore is a substance with unknown or variable composition, complex reaction products or biological materials (UVCB) with unspecified molecular and structural formula. The following opinion specifically covers Margosa, ext. of cold-pressed oil of *Azadirachta indica* seeds without shells extracted with super-critical carbon dioxide (hereinafter *Margosa CO_2-ext.*). *Margosa CO2-ext*. is a biocidal active substance approved for use as an insect repellent biocide (PT19). The total content of limonoids is 2.7 ± 0.4% including azadirachtin A (the active substance) derived from kernels. The content of azadirachtin A in *Margosa CO_2-ext.* is much lower than for other extracts, which indicates that the removal of shells in the manufacturing process has an important impact on the amount of azadirachtin A. The main constituents (> 90%) of *Margosa CO_2-ext.* are triglycerides of fatty acids (oleic, stearic and linoleic acid). Different batches of *Margosa CO_2-ext.* (including those used to perform (eco-)toxicological studies) were analysed and it is noted that the concentrations of individual constituents were very similar between them.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance is an active substance in the meaning of Directive 98/8/EC (repealed by Regulation (EU) 528/2012) and shall normally be subject to harmonised classification and labelling, and justification is not required.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

The EINECS entry (EC no. 283-644-7, CAS no 84696-25-3) is a general entry covering all kinds of extracts from *Azadirachta indica,* Meliaceae irrespective of the extraction conditions:

Extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, oleoresins, terpenes, terpene-free fractions, distillates, residues, etc., obtained from Azadirachta indica, Meliaceae.

According to the guidance for identification and naming of substances under REACH and CLP the different extracts get different names. However, the EC name and number is valid for all these extracts. This - CLH dossier was prepared for the following extract:

- Margosa Extract, cold-pressed oil of *Azadirachta indica* seeds without shells extracted with super-critical carbon dioxide

However, extracts can in general also be obtained by using water or other organic solvents for the extraction. There are overall three relevant examples for such an extract:

- Margosa extract from the kernels of *Azadirachta indica* extracted with water and further processed with organic solvents. This extract is already included in the Union list included in the biocide regulation.
- Margosa extract from the kernels of *Azadirachta indica* extracted with organic solvents at elevated temperatures
- Margosa extract from presscake of kernels of *Azadirachta indica* after removal of the Neem oil, extracted with organic solvents at elevated temperatures

Concluding, since now in total four margosa extracts (all covered by the EINECS entry) are known to be on the market. This dossier was prepared for one of these extracts.

Table 5: Substance identity

EC number:	283-644-7
EC name:	Margosa, ext.
CAS number (EC inventory):	84696-25-3
CAS number:	84696-25-3
CAS name:	Margosa, ext.
Name	Margosa Extract, cold-pressed oil of *Azadirachta indica* seeds without shells extracted with super-critical carbon dioxide
IUPAC name:	Not available
CLP Annex VI Index number:	-
Molecular formula:	Not available; substance is an UVCB
Molecular weight range:	Not available; substance is an UVCB

Structural formula:

Not available substance is an UVCB

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Please refer to the confidential Annex for further information			

Since the substance is an UVCB no impurities are assigned.

1.2.1 Composition of test material

100 % w/w Margosa Extract, cold-pressed oil of *Azadirachta indica* seeds without shells extracted with super-critical carbon dioxide (hereinafter *"Margosa Extract"*).

1.3 Physico-chemical properties

Table 7: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	Yellow-brown liquid	Smeykal, 2003a	Organoleptic
Melting/freezing point	The melting range is -16 to + 20 °C under atmospheric pressure.	Smeykal, 2003a	OECD 102 / EC A.1
Boiling point	n.a. (decomposition at 340 °C)	Smeykal, 2003a	OECD 103 / EC A.2
Relative density	relative density 0.92501 at 20 °C	Wilfinger, 2003a	OECD 109 / EC A.3 (pycnometer method)
Vapour pressure	3.8 x 10-7 hPa at 20 °C,	Franke, 2005a	92/69/EEC, A.4 (vapour pressure balance)
Surface tension	35.3 mN/m at 20 °C (c = 1g/l)	Wilfinger, 2003	92/69/EC, A.5 (ring method)
Water solubility	azadirachtin: 34516 mg/l; linolic acid: 0.045077 mg/l; α-Linoleic acid: 0.099004 mg/l; oleic acid: 0.020522 mg/l; stearic acid: 0.6 mg/l; Eicosanoic acid: 0.00086554 mg/l		calculation (EPIWIN v.3.12)
	pH 3 10°C:420 [mg/kg] 20°C:430 [mg/kg] 30°C:410 [mg/kg]	Bockholt, 2006	92/69/EEC, A.6 (flask method)

Partition coefficient n-octanol/water	Azadirachtin A : 1.3 Nimbin : 3.0 Salannin : 3.5 all at pH 7 and Azadirachtin A : 1.34 Nimbin : 3.09 Salannin : 3.51 all at pH 5 Azadirachtin A : 1.73 Nimbin : 3.36 Salannin : 3.79 all at pH 9 The fatty acids which are the main components of *Margosa Extract* could not be detected with the used HPLC-system. However, these components are not biologically active and, therefore, of little relevance for the assessment of risks.	Bockholt, 2006	92/69/EC, A.8 (HPLC method)
Flash point	207.8 °C	W.Wilfinger (2003), Report No. 20021424/01-PCFB	92/69/EEC, A.9 (DIN 51758)
Flammability Flammability upon ignition (solids, gases) Flammability in contact with water Pyrophoric properties	Not applicable, substance is a liquid *Margosa Extract* comprises mainly fatty acids bond in glycerides, together with substantial amounts of limonoids. None of the constitutes is known as flammable in contact with water and did show exotherm reaction under normal conditions. This is in line with the long year experience in production, packaging and cleaning of the production equipment.	Margosa Extract, cold-pressed oil of *Azadirachta indica* seeds without shells extracted with super-critical carbon dioxide - Doc IIIA, Subsection A3.11	JUSTIFICATION FOR NON-SUBMISSION OF DATA

Explosive properties	The heat of decomposition in the DSC measurement was far below 500 J/g. Additionally, the ingredients are known to have no explosive properties. The test item has no danger of explosion according to the explosive properties in the sense of Guideline 92/69/EEC, A.14.	H. Smeykal, (2003) Report No. 20021483.02	92/69/EEC, A.14 (DSC)
Auto-ignition temperature (liquids and gases)	395 °C	H. Smeykal, (2003) Report No. 20021483.02	92/69/EEC, A.15 (IEC 79-4 (see DIN 51 794)
Oxidising properties (liquids)	The test item has no oxidizing properties in the sense of the Consolidated version of Council Directive 67/548 EEC Annex V, Method A.21.	J.Franke (2005), Report No. 20050729.01	2004/73/EC, A.21
Corrosive to metals	From the structural formula and composition of the substance it can be concluded that *Margosa Extract* doesn't have to be classified as corrosive to metals.	BAM 3.2	Expert statement No experimental data available.
Granulometry	Not applicable, substance is a liquid		
Stability in organic solvents and identity of relevant degradation products	result: 1,2-dichlorethane: > 250 g/l octanol : > 250 g/l aceton: 80–100 g/l i-propanol: 80–100 g/l temperature:20 °C	Wilfinger, 2003	CIPAC MT 181
Dissociation constant	Not applicable		
Viscosity	result: 0.1202 Pa s temperature:20 °C result: 0.0612 Pa s temperature:40 °C	Wilfinger, 2003	OECD 114 (rotational viscometer

Data waiving

Information requirement: Flammable gases (including chemically unstable gases)

Reason: study technically not feasible

Justification: The study does not need to be conducted because the substance is a liquid.

Information requirement: Aerosols

Reason: study technically not feasible

Justification: The study does not need to be conducted because the substance is no aerosol.

Information requirement: Oxidising gases

Reason: study technically not feasible

Justification: The study does not need to be conducted because the substance is a liquid.

Information requirement: Gases under pressure

Reason: study scientifically unjustified

Justification: The study does not need to be conducted because the substance is a liquid.

Information requirement: Flammable solids

Reason: study technically not feasible

Justification: The study does not need to be conducted because the substance is a liquid.

Information requirement: Self-reactive substances and mixtures

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because there are no chemical groups present in the molecule which are associated with explosive or self-reactive properties and hence, the classification procedure does not need to be applied.

Information requirement: Pyrophoric liquids

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because the substance is known to be stable in contact with air at room temperature for prolonged periods of time (days) and hence, the classification procedure does not need to be applied.

Information requirement: Pyrophoric solids

Reason: study technically not feasible

Justification: study technically not feasible

Information requirement: Self-heating substances and mixtures

Reason: study technically not feasible / study scientifically not necessary

Justification: The study does not need to be conducted because the substance is a liquid.

Information requirement: Substances and mixtures which in contact with water emit flammable gases

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because the experience in production or handling shows that the substance does not react with water, e.g. the substance is manufactured with water or washed with water.

Information requirement: Oxidising solids

Reason: study technically not feasible

Justification: The study does not need to be conducted because the substance is a liquid.

Information requirement: Organic peroxides

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because the substance does not fall under the definition of organic peroxides according to GHS and the relevant UN Manual of tests and criteria.

2 MANUFACTURE AND USES

2.1 Manufacture

Margosa Extract is manufactured using cold-pressed oil of *Azadirachta indica* seeds extracted with super-critical carbon dioxide.

(For further information on the manufacture of the substance please refer to the confidential annex.)

2.2 Identified uses

The substance is used as an active substance in the meaning of Directive 98/8/EC (repealed by Regulation (EU) 528/2012).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 8: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 9			

3.1 Summary and discussion

A flash point of 207.8 °C was determined according to the standard DIN 51758 (92/69/EEC, A.9).

Experience in handling and use indicates *Margosa Extract* is not pyrophoric and does not react with water to liberate flammable gases.

Further, it was also tested in a standard auto-ignition temperature study (92/69/EEC, A.15) and spontaneous ignition was found at 395 °C.

A study for self-heating substances/mixtures does not need to be conducted because the substance is a liquid.

As a screening method for the determination of explosive properties differential scanning calorimetry's (DSC) were performed. The two DSC-measurements showed exothermal effects in the temperature range 340 - 450 °C with a decomposition energy of 110 J/g and 52 J/g, respectively. Therefore explosive properties are excluded.

A test according to the EEC Method A.21 was performed. Due to the fact that the 1:1 mixture, by mass, of test item and cellulose has a mean pressure rise time higher than that of a 1:1 mixture, by mass, of 65 % nitric acid and cellulose the test item has no oxidizing properties in the sense of EEC Method A.21.

No experimental data available to assess the hazard class corrosive to metals. From the structural formula and composition of the substance it can be concluded that *Margosa Extract* doesn't have to be classified as corrosive to metals.

3.2 Comparison with criteria

Margosa Extract does not have to be classified as flammable liquid because the flash point is higher than 60 °C.

The low decomposition energy from DSC-measurements indicated that *Margosa Extract* does not have to be classified as explosive or self-reactive substances and mixtures.

The test results of EEC Method A.21 are sufficient to evaluate the oxidising properties in accordance with Regulation (EC) No 1272/2008.

3.3 Conclusions on classification and labelling

No classification and labelling with regard to the physical hazards are proposed.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Margosa CO_2-ext. is not a flammable liquid. Furthermore, the classification for pyrophoric liquids is not considered applicable, as the substance is known to be stable in contact with air at room temperature for prolonged periods of time. The substance has no oxidising or explosive properties according to results of method A.21 (67/548 EEC, Annex V) and of method A.14 (92/69/EEC), respectively. Waiving arguments have been provided for the following hazard classes: flammable gases, oxidising gases, gases under pressure, flammable solids, pyrophoric solids/liquids, oxidising solids, flammable aerosols and self-heating substances

Overall, no classification was proposed by the dossier submitter (DS) for the physical hazards.

Comments received during public consultation

No comments were received on physical hazards.

Assessment and comparison with the classification criteria

The tests conducted according to the methods A.14 (explosive), A.21 (oxidising), and A.15 (auto-ignition) demonstrate that *Margosa CO_2-ext.* is not explosive, oxidising or auto-flammable. Moreover, *Margosa CO_2-ext.* comprises mainly fatty acids and limonoids, and none of the constituents is known to be flammable in contact with water (nor show exothermic reaction under normal condition) indicating that *Margosa CO_2-ext.* is not highly flammable.

RAC agrees with the DS that **classification for physical hazards is not warranted**.

4 HUMAN HEALTH HAZARD ASSESSMENT

Margosa Extract is a CO_2-extract derived from cold-pressed neem seed oil without shells (*Azadirachta indica*) using the manufacturing method developed by the applicant. *Margosa Extract* acts as a repellent against worker ants. As a botanical extract it belongs to the group of substances with unknown or variable composition, complex reaction products or biological (UVCB) with unspecified molecular and structural formula. The total content of limonoids was determined to be 2.7 ± 0.4 % including azadirachtin A. *Margosa Extract* in this dossier is considered different in composition and properties from other Margosa extracts (e.g. NeemAzal, Fortune Aza, ATI-720 = NPI 720) (CLH dossiers published for commenting on ECHA homepage in October 2014). This does also account for the content of aflatoxins which is much lower in *Margosa Extract*. *Margosa Extract* is, therefore, considered as another substance.

Consequently, studies performed with one of the above-mentioned extracts are not considered in this dossier and read across to those extracts is considered not applicable. Likewise, toxicity studies with neem products found in the open literature were considered not relevant for *Margosa Extract* due to different starting material or extraction procedures.

Short summaries of the available data are included below, which were extracted from "Doc IIA" prepared for the biocidal procedure. More extensive (robust) study summaries are included in the attached "Doc IIIA6" also prepared for the biocidal procedure.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Table 9: Summary of toxicokinetic studies

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results (excretion via respiration, urine, faeces, bile, half-life time plasma, residues in tissue)	Remarks	Reference
No study submitted - Justification for non-submission accepted						

4.1.1 Summary and discussion on toxicokinetics

No studies on absorption, distribution, metabolism and excretion were submitted. Such ADME studies are usually performed with radioactively labelled compounds. However, *Margosa Extract* is a plant-derived oily substance and contains many known but also unknown constituents. In order to obtain a homogeneously labelled extract it would be necessary to grow the tree in a radioactive environment. The active compounds of the neem kernels are not known; the triterpenoids (known as limonoids) and among them the azadirachtins are supposedly the most relevant in effectiveness against insects. Generally, azadirachtin A is treated as the lead compound of extracts prepared from neem seeds but it is unknown, if this substance is also the most relevant with regard to toxicological aspects. In the open literature it was reported on the production of radioactive azadirachtin and on the incorporation of [2-^{14}C] mevalonic acid into azadirachtin in seed kernels and homogenate (Akhila et al., 1998). However, neither azadirachtin A nor any other limonoid is available as radioactive compound in larger amounts for ADME studies. Based on lack of technical feasibility, it is considered acceptable that no studies on metabolism and toxicokinetics were submitted for the biocidal procedure.

Margosa Extract contains only small amounts of limonoids. As the active substance is a complex mixture of various compounds, *Margosa Extract* is regarded as active substance in accordance with the "*Guidance Document on Botanical Active Substances Used in Plant Protection Products*" (SANCO/11470/2012- rev.8, 20 March 2014).

4.2 Acute toxicity

Table 10: Summary of acute toxicity studies

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels	Value LD50/LC50 Main effects	Remarks	Reference
OECD 423	Oral, gavage	Rat, Sprague-Dawley, 3 M + 3 F	2000 mg/kg bw	LD_{50}: > 2000 mg/kg bw No toxic signs observed	Test substance: *Margosa Extract*, Batch 420003	Chevalier F, 2003 LPT Report No. 16315/02
OECD 402	Dermal	Rat, Sprague-Dawley, 5 M + 5 F	2000 mg/kg bw	LD_{50}: > 2000 mg/kg bw No toxic signs observed	Test substance: *Margosa Extract*, Batch 420003	Chevalier F, 2003 LPT Report No. 16316/02
OECD 403	Inhalation Nose only	Rat, Sprague-Dawley, 5 M + 5 F	5.15 mg/L	LC_{50}: > 0.82 mg/L No toxic signs observed	MMAD 8.75 ± 3.87, respirable fraction 0.82 mg/L Test substance: *Margosa Extract*, Batch 420003	Chevalier F, 2003 LPT Report No. 16317/02

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In a limit test, *Margosa Extract* was administered by oral gavage to three adult Sprague-Dawley rats of each sex at a dose of 2000 mg/kg bw. No mortality or any other toxic reaction occurred. No abnormalities were found in the animals upon macroscopic *post mortem* examination 15 days after the treatment. There was no significant effect on body weight. The oral LD_{50} value of *Margosa Extract* in rats was established as exceeding 2000 mg/kg bw.

4.2.1.2 Acute toxicity: inhalation

In an acute inhalation toxicity study, groups of adult Sprague-Dawley rats (5/sex) were exposed by nose-only inhalation to an aerosol of *Margosa Extract* for 4 hours at an actual concentration of 5.15 mg/L air which was the highest achievable concentration, limited by the nature of the test substance. The mass median aerodynamic diameter in the particulate aerosol was 8.75 µm and the concentration

of particles with a respirable size was found to be only 0.82 mg/L. Under the conditions of this experiment *Margosa Extract* caused no mortality. Toxicological symptoms could not be observed during a 14-day observation period. Post mortem findings did not show any macroscopic organ changes. The 4-hour inhalation LC_{50} of *Margosa Extract* for male and female rats exceeded 0.82 mg/L air (the respirable fraction).

4.2.1.3 Acute toxicity: dermal

In an acute dermal toxicity limit study, five adult Sprague-Dawley rats of each sex were exposed to *Margosa Extract* by the dermal route. Test material was applied for 24 hours to 10 % of each animal's body surface (30 cm^2) at a dose of 2000 mg/kg bw. Animals were observed for the following 15 days. No mortality occurred. No clinical signs of systemic toxicity were noted. The mean body weight gain during the observation period was within the range expected for rats used in this type of study. No abnormalities were found at macroscopic post mortem examination of the animals. The dermal LD_{50} of *Margosa Extract* in rats was > 2000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

No data submitted by the applicant.

4.2.2 Human information

Human information is available from poisoning incidents following oral ingestion of "Margosa Oil", which is used as a traditional medicine in Asia and Africa. Case reports (Table 30) describe severe intoxications in children predominantly following oral administration of "Margosa Oil" as a home remedy for the treatment of various diseases (e.g. common cold, deworming). Vomiting, drowsiness, convulsions, metabolic acidosis, and encephalopathy are among the reported signs of poisoning, autopsy of fatal cases revealed liver damage. According to some authors, the findings resemble those of Reye's syndrome (e.g. Sinniah and Baskaran 1981, Sinniah et al. 1981, Sundaravalli et al. 1982). Most of the cases of acute poisoning were reported from the use of unrefined and not standardised home remedies lacking any quality control and containing unknown quantities of toxic substances genuine to the seeds or other parts of the neem tree. In addition, contamination with aflatoxins and/or other harmful compounds may contribute to the toxic profile of the ingested home remedies (e.g. Sinniah and Baskaran 1981, Sinniah et al. 1981, Niemann 2002). One case of suicidal intake of the pesticide NeemAzal-T/S (Parry Agro Ltd, Chennai, India; 1 % azadirachtin, 51 % vegetable oil, 45 % tensides) was reported from a 35-year old woman without evidence of renal or hepatic complications. She recovered completely after intensive care without long-term sequelae (Yiiadural et al. 2010).

4.2.3 Summary and discussion of acute toxicity

For results in the available studies, c.f., Table 10.

In addition, information on human poisoning incidents following oral ingestion of "Margosa Oil" are available. Nevertheless, the information are of limited relevance for classification and labelling of *Margosa Extract* due to unknown composition as well as different starting material and extraction procedures with unknown content of impurities.

4.2.4 Comparison with criteria

Table 11 presents the relevant CLP criteria. LD50/LC50 values after oral, dermal or inhalative administration were above the threshold levels leading to a classification.

Table 11: CLP criteria for acute toxicity classification

CLP criteria	
Oral	
Cat. 4 (H302):	$300 < LD_{50} \leq 2000$ mg/kg (oral)
Cat. 3 (H301):	$50 < LD_{50} \leq 300$ mg/kg (oral)
Cat. 2 (H300):	$5 < LD_{50} \leq 50$ mg/kg (oral)
Cat. 1 (H300):	$LD_{50} \leq 5$ mg/kg (oral)
Inhalation	
Cat. 4 (H332):	$10.0 < LC_{50} \leq 20.0$ mg/L (vapours) $1.0 < LC_{50} \leq 5.0$ (dusts and mists)
Cat. 3 (H331):	$2.0 < LC_{50} \leq 10.0$ mg/L (vapours) $0.5 < LC_{50} \leq 1.0$ (dusts and mists)
Cat. 2 (H330):	$0.5 < LC_{50} \leq 2.0$ mg/L (vapours) $0.05 < LC_{50} \leq 0.5$ (dusts and mists)
Cat. 1 (H330):	$LC_{50} \leq 0.5$ mg/L (vapours) $LC_{50} \leq 0.05$ (dusts and mists)
Dermal	
Cat. 4 (H312):	$1000 < LD_{50} \leq 2000$ mg/kg (dermal)
Cat. 3 (H311):	$200 < LD_{50} \leq 1000$ mg/kg (dermal)
Cat. 2 (H310):	$50 < LD_{50} \leq 200$ mg/kg (dermal)
Cat. 1 (H310):	$LD_{50} \leq 50$ mg/kg (dermal)

4.2.5 Conclusions on classification and labelling

In summary and based on the submitted data, *Margosa Extract* does not meet the criteria to be classified for oral, dermal or inhalative toxicity according to the criteria of the CLP regulation.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS presented three studies performed in accordance with Good Laboratory Practices (GLP) and OECD Test Guidelines (TG) 423, 402, 403, respectively for acute oral, acute dermal and acute inhalation toxicity.

Oral toxicity

In a limit test, *Margosa CO_2-ext.* was administered by oral gavage to three adult Sprague-Dawley rats of each sex at a dose of 2000 mg/kg bw. No mortality or any other toxic reaction occurred. No abnormalities were found in the animals upon macroscopic post mortem examination 15 days after the treatment. There was no significant effect on body weight. The oral LD_{50} value of *Margosa CO_2-ext.* in rats was established as exceeding 2000 mg/kg bw.

In addition, information on human poisoning incidents following oral ingestion of "Margosa Oil" are available. The information is, however, of limited relevance since the composition

of the respective "Margosa oil" is to a large extent unknown, and different starting material with different extraction procedures have been used. "Margosa oil" or "Neem oil" is used as a traditional medicine in Asia and Africa for the treatment of various diseases (e.g. common cold, deworming). Vomiting, drowsiness, convulsions, metabolic acidosis, and encephalopathy are among the reported signs of poisoning. Most of the cases were reported from the use of unrefined and not standardised home remedies lacking any quality control and containing unknown quantities of toxic substances (genuine to the seeds or other parts of the neem tree). For details see the Background Document (Tables 29 and 30, pp. 41-43).

Inhalation toxicity

In an acute inhalation toxicity study, groups of adult Sprague-Dawley rats (5/sex) were exposed by nose-only inhalation to an aerosol of *Margosa CO_2-ext.* for 4 hours at an actual concentration of 5.15 mg/L air which was the highest achievable concentration, limited by the nature of the test substance. The mass median aerodynamic diameter (MMAD) in the particulate aerosol was 8.75 µm and the concentration of particles with a respirable size was found to be only 0.82 mg/L. Under the conditions of this experiment *Margosa CO_2-ext.* caused no mortality. Toxicological symptoms could not be observed during a 14 day observation period. Post mortem examination did not show any macroscopic organ changes. The 4 hour inhalation LC_{50} of *Margosa CO_2-ext.* for male and female rats exceeded 0.82 mg/L air (the respirable fraction).

Dermal toxicity

In an acute dermal toxicity limit study, five adult Sprague-Dawley rats of each sex were exposed to *Margosa CO_2-ext.* by the dermal route. Test material was applied for 24 hours to 10% of each animal's body surface (30 cm^2) at a dose of 2000 mg/kg bw. Animals were observed for the following 15 days. No mortality occurred. No clinical signs of systemic toxicity were noted. The mean body weight gain during the observation period was within the range expected for rats used in this type of study. No abnormalities were found at macroscopic post mortem examination of the animals. The dermal LD_{50} of *Margosa CO_2-ext.* in rats was >2000 mg/kg bw.

The DS concluded that *Margosa CO_2-ext.* did not warrant classification for acute oral, dermal or inahalation toxicity.

Comments received during public consultation

One comment from a Member State Competent Authority (MS) referred to the oral dose applied in the acute toxicity study (2000 mg/kg bw) that according to that MS cannot directly be used to conclude on a lack of poisoning in humans at this dose. The DS agreed to the comment and highlighted that medical observational data on workers support that *Margosa CO_2-ext.* has no potential for acute toxicity.

Assessment and comparison with the classification criteria

Oral

Classification is required where the LD_{50} is ≤ 2000 mg/kg bw based on results from animal studies. The acute oral LD_{50} for *Margosa CO_2-ext.* is > 2000 mg/kg bw in the rat and thus does not require classification.

Beside the acute oral toxicity study, the DS summarised data from human poisoning incidents. Indeed, "Margosa Oil" is used as a traditional medicine in Asia and Africa to cure various diseases (e.g. cold, deworming). In the background document (pp. 41-43, Tables 29 and 30) these poisoning incidents are described and show that the accidental ingestion of "Margosa Oil" may lead to signs of vomiting, drowsiness, convulsions, metabolic acidosis, and encephalopathy.

RAC notes that no specific human poisoning case is reported for *Margosa CO_2-ext.* (covered by the present classification proposal). Since varying extractions methods, starting material (e.g. seeds, bark) and impurities clearly lead to different compositions, the poisoning incidents summarised by the DS do not constitute relevant information to be taken into account for classification purposes.

Inhalation

Classification is required where the LC_{50} value of ≤ 5 mg/L (dusts and mists). The highest achievable concentration was 5.15 mg/L air. The concentration of particles with a respirable size was found to be only 0.82 mg/L. This concentration did not cause mortality in rats and no toxicological symptoms were observed during a 14 day observation period. Thus, the 4h LC_{50} (dust/solid aerosols) to rats for *Margosa CO_2-ext.* is > 0.82 mg/l, which is reported to be the maximum technically achievable concentration.

Dermal

Classification is required where the LD_{50} is ≤2000 mg/kg bw. The LD_{50} in rats was > 2000 mg/kg bw.

Conclusion

RAC agrees with the DS that **no classification is warranted for acute oral, dermal or inhalation toxicity**.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

See section 4.2 for results of acute toxicity studies. No non-lethal effects were reported after acute exposure of *Margosa Extract* via oral, inhalative or dermal route, including clinical signs, influence on behaviour, effects on body weight gain or changes in macroscopic examination. Concerning respiratory tract irritation or narcotic effects, no specific studies (conducted in non-humans or

humans) are available. In the acute inhalation study in rats, no clinical signs, inhibition of body weight gain or necropsy findings were reported. Neither histopathological findings nor practical observations in humans are available. However, the lack of respiratory signs in the acute inhalation study with rats and the lack of effects in the eye irritation study with rabbits argue against a potential of *Margosa Extract* to induce respiratory irritation.

4.3.2 Comparison with criteria

Table 12: Classification criteria for Categories 1, 2 and 3 of specific target organ toxicity-single exposure (C: guidance value)

CLP criteria	
Category 1 (H370) Oral (rat): C ≤ 300 mg/kg bw Dermal (rat or rabbit): C ≤ 1000 mg/kg bw Inhalative (rat, dust/mist/fume): ≤ 1 mg/L/4 h	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure - reliable and good quality evidence from human cases or epidemiological studies; or - observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations.
Category 2 (H371) Oral (rat): 2000 ≥ C > 300 mg/kg bw Dermal (rat or rabbit): 2000 ≥ C > 1000 mg/kg bw Inhalative (rat, dust/mist/fume): 5 ≥ C > 1 mg/L/4 h	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure - observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.
Category 3 (H335/H336) Guidance values do not apply (mainly based on human data). Moreover, no effects relating to changes in respiratory pattern were reported in any inhalation study.	Transient target organ effects This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

4.3.3 Conclusions on classification and labelling

Considering that no non-lethal effects were reported after acute exposure or reported effects were of no considerably adverse nature with no significant impact on health, no classification with STOT-SE 1/2 is proposed. In addition, based on the submitted data, *Margosa Extract* does not meet the criteria to be classified as STOT-SE 3 for respiratory tract irritant or narcotic effects.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS did not propose to classify *Margosa CO_2-ext*. as STOT SE 1 or 2 considering that non-lethal adverse effects were not reported after acute exposure. In addition, based on the submitted data, the DS concluded that *Margosa CO_2-ext.* does not meet the criteria to be classified as STOT SE 3 for respiratory tract irritant or narcotic effects.

Comments received during public consultation

No comment was received during public consultation.

Assessment and comparison with the classification criteria

No signs of organ toxic effects were observed in the acute oral, dermal or inhalation toxicity studies with rats exposed to *Margosa CO_2-ext*. The animal data submitted did not provide evidence for respiratory tract irritation or narcotic effects.

Information on human poisoning incidents following oral ingestion of "Margosa Oil" are considered by RAC as of limited relevance as explained in the section above. Besides the human poisoning, data from medical observations on workers involved in the production of the Margosa extract were negative over a three-year observation period.

Based on this information RAC agrees with DS that **no classification is warranted for STOT SE**.

4.4 Irritation

4.4.1 Skin irritation

Table 13: Summary of skin irritation studies

ö	Species, Strain, Sex, No/group	Average score for each animal (mean: 24, 48, 72 h)		Reversibility yes/no	Results	Remarks	Reference
		Erythema	Edema				
OECD 404	Rabbit, Himalayan, 3 M	0,0,0	0,0,0	Not applicable	Not irritating	Test substance: *Margosa Extract* Batch: 420003	Leuschner J, 2003 LPT Report No. 16318/02
OECD 410 Dermal, semi-occlusive, 28 d	Rat, Hsd: SD, 5 M + 5 F Doses: 0, 100, 500, 1000 mg/kg bw/d	for study details see section 4.7.1.3; Table 20		yes	Local erythema, slight to severe at ≥500 mg/kg bw/d	Skin irritation transient, reversible under treatment during week 2 Test substance: *Margosa Extract*, Batch: 040515	Cicalese R, 2005 RTC Report No. 44070
OECD 414 Dermal, semi-occlusive, GD 6-28	Rabbit, NZW, 35 F Doses: 0, 50, 200, 800 mg/kg bw/d	for study details see section 4.7.1.3; Table 21		no	Local irritation considered adverse ≥200 mg/kg bw; systemic: bw gain ↓ at 200 and 800 mg/kg bw, not considered adverse	Test substance: Batch: *Margosa Extract*, 040515	Cicalese R, 2006 RTC Report No. 44800

4.4.1.1 Non-human information

In a primary dermal irritation study, three male Himalayan rabbits were exposed via the dermal route to 0.5 mL of *Margosa Extract* each. The test material was applied for 4 hours to the clipped skin of the back, using a semi-occlusive dressing. No symptoms of systemic toxicity were found and no mortality occurred. Exposure to *Margosa Extract* did not result in any skin reactions. Based on these results, *Margosa Extract* is not regarded as a skin irritant.

In addition, two studies with dermal application (28-d in rats and prenatal toxicity in rabbits) should be further considered when assessing skin corrosion and irritation of *Margosa Extract*.

In a 28-d rat dermal study with *Margosa Extract*, no systemic effects were observed. Slight to well-defined erythema with or without desquamation was noted in all males and females receiving 500 mg/kg/day towards the end of the first week of dosing (days 5-7). In the dose groups receiving 1000 mg/kg/day incidence and time of appearance were similar (days 5-8) and the grading ranged from slight to severe (Table 20). The skin irritation disappeared in both groups during the second week of dosing and no further changes became apparent after that point in time.

Furthermore, local skin irritating effects were observed in a prenatal toxicity study in rabbits in all treated dose groups and were considered adverse from 200 mg/kg bw/d onwards. However, irritation scores in the lowest dose were low with only a few females affected. The number of females with irritation and the observed scores for irritation and oedema were clearly below classification criteria for skin irritation (the latter related to acute exposure). Therefore, the slight irritating effects in the lowest dose group (50 mg/kg bw/d) were not regarded as adverse. Moderate local skin effects with persistent erythema and oedema were observed after application of 200 mg/kg bw/d *Margosa Extract* at the end of the study. Very slight erythema/oedema appeared on day 2/5 of treatment in one female whilst on day 16, erythema (with an average score of 1.90) were evident in all animals. Further skin changes in a few animals in consequence of treatment were desquamation, fissuration and scabs. At the highest concentration (800 mg/kg bw/d) very slight erythema appeared after single application in one female. Persistent erythema (average score: 2.56) and oedema (average score: 2.71) were evident in all females from day 16 onwards. With prolonged treatment erythema and oedema turned out severe in individual females. These effects were accompanied by desquamation, fissuration and scabs. The macroscopic examination at terminal sacrifice revealed a dose related increase of red coloration and scabs in a few animals.

4.4.1.2 Human information

No human information submitted by the applicant.

4.4.1.3 Summary and discussion of skin irritation

In the available dermal irritation study in rabbits no symptoms of systemic toxicity were found and no mortality occurred. Exposure to *Margosa Extract* did not result in any skin reactions.

However, data from a 28-day study in rats and a prenatal toxicity study in rabbits with dermal application indicate that *Margosa Extract* can induce skin irritation after approximately five (rats) to ten (rabbits) days of dosing. In rats, dose-dependent, slight to severe erythema with and without desquamation was observed transiently for about 3-4 days, but resolved spontaneously despite continuing treatment. In rabbits, the effects were dose-dependent as well and continued to be present for the duration of the study at the two highest doses. After single application of 800 mg/kg bw/d *Margosa Extract* to female rabbits, only one of a total of 20 females showed very slight erythema, which is not considered sufficient for classification and labelling as a skin irritant.

In addition, labelling with EUH066 – Repeated exposure may cause skin dryness or cracking – is not proposed because the observed effects were not dryness of the skin. As *Margosa Extract* has a high content of fatty acids, dryness of the skin is not to be expected.

4.4.1.4 Comparison with criteria

Table 14: Results of skin irritation studies in comparison with CLP criteria

Toxicological result	**CLP criteria**
Mean erythema and oedema scores (24-72 h): 0.0 and 0.0, respectively (no animal ≥ 0) Mean erythema and oedema scores (24-72 h): no animal ≥ 0, respectively	Irritating to skin (Category 2, H315): at least in 2/3 tested animal a positive response of: Mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema

In skin irritation studies no scores exceeding 0 were observed for erythema and oedema. Skin findings in dermal rat studies with repeated administration were transient despite of continuing treatment. The local skin effects determined after repeated exposure in rabbits were considered to be irritation for the highest dose with persistent erythema and oedema. Applied in a single dose in the 28-d dermal and prenatal toxicity study, *Margosa Extract* does not meet the criteria for irritating or even corrosive effects to be classified for skin corrosion or irritation.

4.4.1.5 Conclusions on classification and labelling

In summary and based on the submitted data, *Margosa Extract* does not meet the criteria to be classified for skin irritation/corrosion according to the criteria of the CLP regulation.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS provided results from a dermal irritation study conducted according to OECD TG 404. Three male Himalayan rabbits were exposed via the dermal route to 0.5 mL of *Margosa CO_2-ext.* each. The test material was applied for 4 hours to the clipped skin of the back, using a semi-occlusive dressing. No symptoms of systemic toxicity were found and no mortality occurred. Exposure to *Margosa CO_2-ext.* did not result in any skin reactions. Based on these results, *Margosa CO_2-ext.* is not regarded as a skin irritant.

Furthermore, additional repeated dose toxicity studies conducted via the dermal route are summarised by the DS.

In a 28-day study, rats were exposed dermally to *Margosa CO_2-ext*. at dose levels of 100, 500, 1000 mg/kg bw/d (semi-occlusive). A dose-dependent slight to severe erythema with and without desquamation was observed transiently for about 3-4 days, but resolved spontaneously despite continuing treatment.

In a pre-natal developmental toxicity study (PNDT), rabbits were exposed dermally to *Margosa CO_2-ext.* at dose levels of 0, 50, 200, 800 mg/kg bw/d (semi-occlusive). The effects were also dose-dependent and continued to be present for the duration of the study at the two highest doses (200 and 800 mg/kg bw/d). After the first application of 800 mg/kg bw/d (corresponding to 4,5 mg/cm^2) *Margosa CO_2-ext.* to female rabbits, only one of a total of 20 females showed very slight erythema. The DS did not consider these effects as sufficient for classification and labelling as a skin irritant.

In addition, labelling as EUH066 (repeated exposure may cause skin dryness or cracking) was not relevant according to the DS because the observed effects were not characterised as dryness of the skin. *Margosa CO_2-ext.* has a high content of fatty acids, therefore dryness of the skin is not expected.

In summary and based on the submitted data, the DS concluded that *Margosa CO_2-ext.* does not meet the criteria for skin irritation/corrosion according to the criteria of the CLP Regulation. These repeated dose toxicity studies will be further discussed in the section on repeated dose toxicity.

Comments received during public consultation

No comment was received during public consultation.

Assessment and comparison with the classification criteria

Skin irritation means the production of reversible damage to the skin following the application of a test substance for up to 4 hours.

In a standard skin irritation assay in which rabbit skin was exposed to 0.5 mL *Margosa CO_2-ext.* for 4 hours, no skin reaction was observed. In the repeated dose toxicity studies the irritation effects became apparent after repeated application. Skin irritation findings in dermal rat studies were transient despite of continuing treatment. In rabbits, the effect were dose-dependent and continued to be present for the duration of the study. After first application of 800 mg/kg bw (corresponding to 4.5 mg/cm^2) only one out of 20 females showed very slight erythema, which is not considered to be sufficient for classification.

Therefore, RAC concurs with the DS that *Margosa CO_2-ext.* **does not warrant classification for skin irritation**.

4.4.2 Eye irritation

4.4.2.1 Non-human information

Table 15: Summary of eye irritation studies

Method/ Guideline	Species, Strain, Sex, No/group	Average Score for each animal (mean: 24, 48, 72h)				Reversibility yes/no	Results	Remarks	Reference
		Cornea	Iris	Redness Conjunctiva	Chemosis				
OECD 405	Rabbit, Himalayan, 3 M	0,0,0	0,0,0	0,0,0	0,0,0	Not applicable	Not irritating	Grade 1 corneal opacity observed in 2/3 animals at 1 h Test substance: *Margosa Extract* Batch: 420003	Leuschner J, 2003 LPT Report No. 16319/02

4.4.2.2 Human information

No human information submitted by the applicant.

4.4.2.3 Summary and discussion of eye irritation

In a primary eye irritation study, 0.1 mL of *Margosa Extract* was instilled into the conjunctival sac of the right eyes of three adult male Himalayan rabbits. The test substance did not cause any acute systemic toxicological signs or mortality. Instillation of the test substance resulted in grade 1 corneal opacity in two of the three animals 1 h after application. These effects had resolved within 24 hours. Based on these results, *Margosa Extract* is not regarded as an eye irritant.

4.4.2.4 Comparison with criteria

Table 16: CLP criteria for eye irritation

CLP criteria
Irritating to eyes (Category 2, H319): at least in 2/3 tested animal a positive response of: corneal opacity: ≥ 1 and/or iritis: ≥ 1 and/or conjunctival redness: ≥ 2 and/or conjunctival oedema (chemosis): ≥ 2 - calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days

Margosa Extract technical extracts exhibited very slight and reversible irritating potential to eye. According to the study reports, the severity of findings did not reach the critical thresholds to be classified as eye irritant.

4.4.2.5 Conclusions on classification and labelling

In summary and based on the submitted data, *Margosa Extract* does not meet the criteria to be classified for eye irritation/corrosion according to the criteria of the CLP regulation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritation potential of *Margosa CO_2-ext.* was tested in a standard guideline study (OECD TG 405) in which 0.1 mL of *Margosa CO_2-ext.* was instilled into the conjunctival sac of the right eyes of three adult male Himalayan rabbits. The test substance did not cause clinical signs or mortality but resulted in a transient grade 1 corneal opacity in two out of three animals 1 h after application and had resolved within 24 hours. Based on these results, *Margosa CO_2-ext.* is not regarded as an eye irritant.

Margosa CO_2-ext. exhibited very slight and reversible irritating potential to the eye. According to the study reports, the severity of findings did not reach the critical thresholds to be classified as eye irritant according to the DS.

Comments received during public consultation

No comment was received during public consultation.

Assessment and comparison with the classification criteria

A substance should be classified for reversible eye effects (Category 2) if, in at least two of three tested animals, a positive response is observed of corneal opacity ≥1 and/or iritis ≥1 and/or conjunctival redness ≥2 and/or conjunctival oedema ≥2; calculated as mean score following grading at 24, 48 and 72 hours and which are fully reversible.

The findings of the eye irritation study demonstrate that two of three tested animals showed grade 1 corneal opacity 1 hour after application. These effects resolved after 24 hours. Therefore, *Margosa CO_2-ext.* exhibits very slight and reversible irritation potential. However, the criteria to classify *Margosa CO_2-ext.* for eye damaging/irritating effects are not met.

RAC concurs with the DS´s proposal that *Margosa CO_2-ext.* **does not require classification for serious eye damage or for eye irritation**.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No specific studies (conducted in non-humans or humans) concerning respiratory tract irritation were available. In the acute inhalation study in rats, no clinical signs, inhibition of body weight gain or necropsy findings were reported. Neither histopathological findings nor practical observations in humans are available. However, the lack of respiratory signs in the acute inhalation study with rats and the lack of effects in the eye irritation study with rabbits argue against a potential of *Margosa Extract* to induce respiratory irritation.

4.4.3.2 Human information

No human information submitted by the applicant.

4.4.3.3 Summary and discussion of respiratory tract irritation

While no specific data regarding this endpoint were submitted, the available data do not indicate a potential for respiratory tract irritant of *Margosa Extract*.

4.4.3.4 Conclusions on classification and labelling

In summary and based on the submitted data, *Margosa Extract* does not meet the criteria to be classified as respiratory tract irritant.

4.5 Corrosivity

No specific studies regarding corrosion were submitted. Corrosion was not seen in the studies for dermal or eye irritation. Hence, no classification for corrosion of skin or eye is needed. Please compare also section 0 (

Irritation).

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 17: Summary of sensitisation studies

Method/ Guideline	Species, Strain, Sex, No/group	Number of animals sensitised/ total number of animals	Results	Remarks	Reference
OECD 406 (M&K)	Guinea pig, Dunkin-Hartley, 7 F Pretest 20 F Test group 10 F Control	0/20 (HCA control: 10/10)	Not sensitising	Test substance: *MARGOSA EXTRACT* Batch: 040515	Salvador M, 2006 RTC Report No. 49060

4.6.1.1 Non-human information

In a test for dermal sensitisation according to Magnusson and Kligman, 20 young adult female albino guinea pigs were intradermally injected with 50 % (w/v; vehicle: coconut oil) of *Margosa Extract* with Freund's Complete Adjuvant and dermally exposed to 50 % (w/v, vehicle coconut oil) *Margosa Extract*. Ten control animals were treated similarly, but with vehicle alone. Two weeks after the epidermal application, all animals were challenged with 50 % *Margosa Extract* in coconut oil. In this study, *Margosa Extract* produced no evidence of skin sensitisation.

4.6.1.2 Human information

No human information submitted by the applicant.

4.6.1.3 Summary and discussion of skin sensitisation

In the available study, *Margosa Extract* produced no evidence of skin sensitisation.

4.6.1.4 Comparison with criteria

Table 18 presents the toxicological results in comparison with the CLP criteria.

Table 18: Results of skin sensitisation tests in comparison with CLP criteria

Toxicological result	CLP criteria
0/20 animals positive 50 % intra dermal induction concentration	Guinea pig maximisation test Category 1A (H317): ≥ 30 % responding at ≤ 0.1 % intradermal induction dose or ≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose Category 1B (H317): ≥ 30 % to < 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose

4.6.1.5 Conclusions on classification and labelling

In summary and based on the submitted data, *Margosa Extract* does not meet the criteria laid down in the CLP regulation (as amended) to be classified as Skin sensitisation category 1 (H317 - May cause an allergic skin reaction).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

In a test for dermal sensitisation according to Magnusson and Kligman (OECD TG 406), 20 young adult female albino guinea pigs were intradermally injected with 50% (w/v; vehicle: coconut oil) of *Margosa CO_2-ext.* with Freund's Complete Adjuvant and dermally exposed to 50% (w/v, vehicle coconut oil) *Margosa CO_2-ext*. Ten control animals were treated similarly, but with vehicle alone. Two weeks after the epidermal application, all animals were challenged with 50% *Margosa CO_2-ext.* in coconut oil. In this study, *Margosa CO_2-ext.* produced no evidence of skin sensitisation. The DS did not propose to classify *Margosa CO_2-ext*. for skin sensitisation.

Comments received during public consultation

No comments received.

Assessment and comparison with the classification criteria

No signs of sensitisation were seen in the Magnusson and Kligman study according to TG 406. The doses applied were in accordance with OECD TG 406, i.e. the concentration of *Margosa CO_2-ext*. used for induction exposure caused moderate skin irritation and the challenge exposure was a non-irritant dose.

Since no signs of sensitisation were observed, RAC agrees with the DS that **no classification for skin sensitisation is warranted**.

4.6.2 Respiratory sensitisation

No data/information (from non-humans or humans) were submitted that would allow an evaluation of sensitising properties for the respiratory tract.

4.7 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.7.1 Non-human information

Table 19: Summary of repeated dose toxicity studies

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels	NO(A)EL ppm (mg/kg bw /d)	LO(A)EL ppm (mg/kg bw /d)	Results Main effects/ Target organs	Remarks	Reference
OECD 407	Oral in feed, 28 d	Rat, Hsd: SD, 5 M + 5 F	0, 102, 520, 1047 mg/kg bw/d in males and 0, 96, 481, 992 mg/kg bw/d in females	1047 males, 992 females	(> 992)	Liver weight ↑ slight, rel. liver weight increases sign. at highest dose (M: <10 % /F: 13 %), reversible (not considered adverse)	Concentration in food adjusted to achieve constant intake; 14-d recovery groups in control and high dose Test substance: *Margosa Extract* Batch: 040515	Cicalese R, 2006 RTC Report No. 43990
OECD 408	Oral in feed, 90 d	Rat, HsdCpb: WU, 10 M + 10 F	0, 145, 436, 962 mg/kg bw/d in males and 0, 147, 442, 979 mg/kg bw/d in females	approx.. 450	approx.960	Liver weight ↑ (absolute: 13.5 % M/F, relative: 14.6 % (M), 18.1 % F)) reversible	Concentration in food adjusted to achieve constant intake; 28-d recovery groups in control and high dose Test substance: *Margosa Extract* Batch: 560205	Ramesh E, 2009 Report No. G5018
OECD 410	Dermal, semi-occlusive, 28 d	Rat, Hsd: SD, 5 M + 5 F	0, 100, 500, 1000 mg/kg bw/d	Local: (100) Systemic: (1000)	Local: (500) Systemic: (> 1000)	Local erythema, slight to severe at ≥ 500 mg/kg bw/d	Skin irritation transient, reversible under treatment during week 2 Test substance: *Margosa Extract*, Batch: 040515	Cicalese R, 2005 RTC Report No. 44070
OECD 414	Dermal, semi-occlusive, GD 6-28	Rabbit, NZW, 35 F	0, 50, 200, 800 mg/kg bw/d	maternal: Local: (50) Systemic: (800)	maternal: Local: (200)	Local irritation considered adverse ≥200 mg/kg bw; systemic: bw gain ↓ at 200 and 800	Test substance: *Margosa Extract* Batch: 040515	Cicalese R, 2006 RTC Report No. 44800

Method/ Guideline	**Route of exposure, Duration**	**Species, Strain, Sex, No/group**	**Dose levels**	**NO(A)EL ppm (mg/kg bw /d)**	**LO(A)EL ppm (mg/kg bw /d)**	**Results Main effects/ Target organs**	**Remarks**	**Reference**
						mg/kg bw, not considered adverse		

4.7.1.1 Repeated dose toxicity: oral

The only finding in a 28-d rat feeding study with *Margosa Extract* was a slight increase of relative liver weight in males at the mid and high dose and in high dose females. This effect is not considered adverse because the increases were below 10 % in males and below 15 % in females and histopathologic correlates were lacking. Moreover, the organ weight increase was reversible within a two-week recovery period.

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.

4.7.1.2 Repeated dose toxicity: inhalation

No data submitted by the applicant.

4.7.1.3 Repeated dose toxicity: dermal

In a 28-d rat dermal study with *Margosa Extract*, no systemic effects were observed. Slight to well-defined erythema with or without desquamation was noted in all males and females receiving 500 mg/kg/day towards the end of the first week of dosing (days 5-7). In the dose groups receiving 1000 mg/kg/day incidence and time of appearance were similar (days 5-8) and the grading ranged from slight to severe. As the examinations prior to application and approximately 1 h (during appication) did not show more severe skin reactions, Table 20 presents the results of local skin effects 6 h after application. The skin irritation disappeared in both groups during the second week of dosing and no further changes became apparent after that point in time.

Table 20: Number of affected animals with clinical signs of local tolerance to the skin of rats in 28-d dermal study with ***Margosa Extract*** at Session 3 = 6 hours after application (after bandage removal)

Day	Sex	Finding	Dose group (mg/kg bw/d)			
			0	100	500	1000
5	m f	Erythema		 1 #slight	5 #slight 5 #slight	2 #slight, 3 #well defined 2 #slight
	m f	Desquamation			3 1	5 1
6	m f	Erythema			4 #slight 4 #slight	4 #well defined, 1 #moderate to severe 2 #slight, 1 #well defined
	m f	Desquamation			4 2	5 1
7	m f	Erythema			2 #slight 3 #slight	4 #slight, 1 #well defined 2 #slight
	m f	Desquamation			2 2	5 1
8	m f	Erythema				3 #slight 3 #slight
	m f	Desquamation				3 1
9	m	Desquamation				2
10	m	Desquamation				2
11	m	Desquamation				2

skin reaction (slight, well defined or moderate to severe)

Furthermore, local skin irritating effects were observed in a prenatal toxicity study in rabbits in all treated dose groups and were considered adverse from 200 mg/kg bw/d onwards. However, irritation scores in the lowest dose were low with only a few females affected. The number of females with irritation and the observed scores for irritation and oedema were clearly below the classification criteria for skin irritation (the latter related to acute exposure). Therefore, the slight irritating effects in the lowest dose group (50 mg/kg bw/d) were not regarded as adverse. Moderate local skin effects with persistent erythema and oedema were observed after application of 200 mg/kg bw/d *Margosa Extract* at the end of the study. Very slight erythema/oedema appeared on day 2/5 of treatment in one female whilst on day 16, erythema (with an average score of 1.90) were evident in all animals. Further skin changes in a few animals in consequence of treatment were desquamation, fissuration and scabs. At the highest concentration (800 mg/kg bw/d) very slight erythema appeared after single application in one female. Persistent erythema (average score: 2.56) and oedema (average score: 2.71) were evident in all females from day 16 onwards. With prolonged treatment erythema and oedema turned out severe in individual females. These effects were accompanied by desquamation, fissuration and scabs. The macroscopic examination at terminal sacrifice revealed a dose related increase of red coloration and scabs in a few animals.

Nevertheless, severity and duration of the irritation in rats and rabbits is not considered sufficient for classification as STOT RE for dermal exposure. Irritant effects observed in the highest dose group are above the concentration required for STOT RE according to the CLP Criteria (highest dose group:

800 mg/kg bw/d, classification for STOT RE 2: 60< C ≤ 600 mg/kg bw/d). In accordance with the Guidance on the Application of the CLP Criteria (ECHA Nov 2013, p 480 *ff*),

"*STOT-RE is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature which significantly impact on health.* …"

As no signs of toxicity were observed in addition to skin irritation, classification for STOT RE for the dermal route is considered not justified.

Table 21: Group mean data for local skin irritation observations in prenatal developmental toxicity study in female rabbits after dermal application

Day of treatment	Finding		Dose groups (mg/kg/d)			
			0	50	200	800
8	Erythema	Average Score*	0	0.66	1.14	2.00
		Incidence (%)	0	52.9	85.7	100
	Oedema	Average Score*	0	0.14	0.49	1.83
		Incidence (%)	0	14.3	35.7	91.4
16	Erythema	Average Score*	0	1.04	1.90	2.56
		Incidence (%)	0	88.6	100	100
	Oedema	Average Score*	0	0.53	1.64	2.71
		Incidence (%)	0	41.4	91.4	100
23	Erythema	Average Score*	0	0.99	1.86	2.66
		Incidence (%)	0	84.3	96.9	100
	Oedema	Average Score*	0	0.37	1.49	2.81
		Incidence (%)	0	31.4	95.4	100

* skin reaction scoring according to DRAIZE

4.7.1.4 Repeated dose toxicity: other routes

No data submitted by the applicant.

4.7.1.5 Human information

No human information submitted by the applicant.

4.7.1.6 Other relevant information

No data submitted by the applicant.

4.7.2 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Only a slight increase of relative liver weight in males at the mid and high dose and in high dose in females was reported for the oral route in a 28-d rat feeding study with *Margosa Extract*. However, according to the CLP regulation, this small elevation could not be regarded as a significant toxic effect, of relevance to human health and it is also not produced at generally moderate exposure concentrations. No systemic effects were reported in the 28-d rat dermal study and severity, reversibility and duration of the irritation at 500 mg/kg bw/d could not justify the classification as STOT RE for dermal exposure. Even if the rabbit is more susceptible for local skin irritation as the rat, the results from the prenatal toxicity study with rabbits do not point to significant organ damage with severe morphological changes following repeated dermal exposure to *Margosa Extract*. As the effects were limited to irritating effects with erythema, oedema, reddening, desquamation, fissuration and scabs, no histopathological changes such as necrosis, ulcers, bleeding or purulent lesions could be demonstrated.

4.7.3 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Table 22 presents the CLP criteria for classification for STOT RE.

Table 22: criteria of specific target organ toxicity – repeated exposure

CLP criteria
Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for different study durations: Oral, rat: 28-day: ≤ 30 mg/kg bw/d 90-day: ≤ 10 mg/kg bw/d 1-yr: ≤ 2.5 mg/kg bw/d 2-yr: ≤ 1.25 mg/kg bw/d Dermal: 28-day: ≤ 60 mg/kg bw/d 90-day: ≤ 20 mg/kg bw/d
Category 2 (H373): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2. Equivalent guidance values for different study durations: Oral, rat: 28-day: 30 < C ≤ 300 mg/kg bw/d 90-day: 10 < C ≤ 100 mg/kg bw/d

1-yr: 2.5 < C ≤ 25 mg/kg bw/d 2-yr: 1.25 < C ≤ 12.5 mg/kg bw/d Dermal: 28-day: 60< C ≤ 600 mg/kg bw/d 90-day: 20 < C ≤ 200 mg/kg bw/d

No severe findings with significant organ damage were observed in rats at dose levels below the respective guidance values in any of the routes oral and dermal. The skin irritating effects reported in rabbits after dermal exposure were also not sufficient for classification and labelling as STOT RE. Hence, it is proposed not to classify for STOT RE.

4.7.4 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Classification for effects seen in repeated-dose studies was considered not necessary.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS summarised two oral feeding studies (28- and 90-day studies) and two dermal toxicity studies (28-day study, prenatal development toxicity study).

Oral

The only altered observation in a 28 day rat feeding study with *Margosa CO_2-ext.* (dose levels: males: 0, 102, 520, 1047 mg/kg bw/d, females: 0,96, 481, 992 mg/kg bw/d) was a slight increase of relative liver weight in males at the mid and high dose levels and in females at the top dose. This effect was not considered adverse by the DS because the increases in liver weight were ≤10% in males and ≤15 % in females and histopathologic correlates were lacking. Moreover, the organ weight increase was reversible within a two-week recovery period.

In the 90 day rat feeding study with *Margosa CO_2-ext.* (dose levels: males: 0, 145, 436, 962 mg/kg bw, females: 0, 147, 442, 979 mg/kg bw), the top dose induced an increase in liver weight in males and females, without any histopathological correlates, which was reversible within the 4 week recovery period. Since liver weight increases were ≥15% in both sexes, the effect was considered adverse by the DS.

However, the DS concluded that these findings did not constitute significant organ damage in line with the CLP criteria since they were not observed in rats at dose levels within the respective guidance values for STOT RE 2 i.e. 30 < C ≤ 300 mg/kg bw/d (28-day study) or 10 < C ≤ 100 mg/kg bw/d (90-day study).

Dermal

In the 28 day rat study with *Margosa CO_2-ext.* (dose levels: 100, 500, 1000 mg/kg bw/d, semi-occlusive exposure, 6 hours per day, 7 days per week, no vehicle), no systemic effects

were observed. Slight to well-defined erythema with or without desquamation were observed in all males and females exposed to 500 mg/kg bw/day towards the end of the first week of application (days 5-7). In the highest dose groups receiving 1000 mg/kg bw/day incidence and time of appearance were similar to the mid-dose group (days 5-8), but the grading ranged from slight to severe. In the table below the skin effects (6 hours after application) are summarised. The skin irritating effects disappeared during the second week of dosing and no further changes became apparent after that time point.

Table: Number of affected animals at day 5-11 six hours after bandage removal (28 d rat study)

Day	**Sex**	**Finding**	**Dose group mg/kg bw/d**			
			0	100	500	1000
5	*M* *F*	*Erythema*	*0*	*1*slight*	*5*slight* *5*slight*	2*slight, 3*well-defined 2*slight
	M *F*	*Desquamation*	*-*	*-*	*3* *1*	5 1
6	M F	Erythema	-	-	4*slight 4*slight	4*well defined, 1*moderate to severe 2*slight, 1*well defined
	M F	Desquamation	-	-	4 2	5 1
7	M F	Erythema	-	-	2*slight 3*slight	4*slight, 1*well-defined 2*slight
	M F	Desquamation	-	-	2 2	5 1
8	M F	Erythema	-	-	-	3*slight 3*slight
	M F	Desquamation	-	-	-	3 1
9	M F	Desquamation			-	2
10	M F	Desquamation			-	2
11	M F	Desquamation			-	2

*Skin reaction (slight, well defined or moderate to severe)

Local skin irritating effects were also observed in a prenatal toxicity study in rabbits in all dose groups (0, 50, 200, 800 mg/kg bw/d, semi-occlusive exposure, 6 hours per day, no vehicle used, 10% of body surface, on day 26-28 post mating) and are considered by DS as adverse from a dose level of 200 mg/kg bw/d onwards. Irritation scores in the lowest dose (50 mg/kg bw/d) were low with only a few females affected. The number of females with irritation and the observed scores for irritation and oedema were clearly below the classification criteria for skin irritation. Therefore, the slight irritating effects in the lowest dose group (50 mg/kg bw/d) were not regarded as adverse. Moderate local skin effects with persistent erythema and oedema were observed after application of 200 mg/kg bw/d *Margosa CO_2-ext.* at the end of the study. Very slight erythema/oedema appeared on day 2 and 5 of treatment in one female whilst on day 16, erythema (with an average score of

1.90) were evident in all animals. Further skin changes in a few animals in consequence of treatment were desquamation, fissuration and scabs. At the highest concentration (800 mg/kg bw/d) very slight erythema appeared after the first application in one female. Persistent erythema (average score: 2.56) and oedema (average score: 2.71) were evident in all females from day 16 onwards. With prolonged treatment erythema and oedema became therefore more severe in individual females. These effects were accompanied by desquamation, fissuration and scabs. The macroscopic examination at terminal sacrifice revealed a dose related increase of red coloration and scabs in a few animals.

Table: Local skin irritation observation in prenatal developmental toxicity study in female rabbits after dermal application

Day of treatment	**Finding**	**Dose groups (mg/kg bw/day)**			
		0	50	200	800
8	Erythema: Score (Incidence %)	0	0.66 (52.9)	1.14 (85.7)	2.00 (100)
	Oedema: Score (Incidence %)	0	0.14 (14.3)	0.49 (35.7)	1.83 (91.4)
16	Erythema: Score (Incidence %)	0	1.04 (88.6)	1.90 (100)	2.56 (100)
	Oedema: Score (Incidence %)	0	0.53 (41.4)	1.64 (91.4)	2.71 (100)
23	Erythema: Score (Incidence %)	0	0.99 (84.3)	1.86 (96.9)	2.66 (100)
	Oedema: Score (Incidence %)	0	0.37 (31.4)	1.49 (95.4)	2.81 (100)

The DS regarded the severity and duration of the irritation in rats and rabbits as being not sufficient for classification as STOT RE for dermal exposure. Irritant effects observed in the highest dose group were above the concentration required for STOT RE 2 according to the CLP Criteria (highest dose group: 800 mg/kg bw/d, guidance value for STOT RE 2: 60 < C ≤ 600 mg/kg bw/d for a 28-day study).

As no signs of toxicity were observed in addition to skin irritation, classification for STOT RE for the dermal route was considered not justified by the DS.

Comments received during public consultation

No comments received during public consultation.

Assessment and comparison with the classification criteria

In the two oral repeated dose toxicity studies in rats, the only findings described are changes in liver weight. In the 28 day study the liver weight change (males: ≤10%, females: 13%, relative) was only observed at levels far above the guidance values for STOT RE 2 classification. This observation was reversible (14 day recovery group) and without histopathological correlates. In the 90 day repeated dose toxicity study liver weight

change was induced in the top dose of approximatively 960 mg/kg bw/d (above 15%). The changes were reversible within the 4 week recovery period and no histopathological correlates were observed. Although, the effects are considered as adverse they occurred only far above the guidance values for STOT RE 2 classification (see Table).

Table: Overview of main findings in repeated dose toxicity studies and comparison with guidance values

Study	**Observed effect**	**Effect level, mg/kg bw/day**	**Guidance values (STOT RE 2), oral rat mg/kg bw/day**
28 day, rat, oral	Increased liver weight: M: ≤10% F: 13%	436, 1047 992	30 < C ≤ 300
90 day, rat, oral	Increased liver weight: M/F: absolute 13.5% M: relative 14.6 % F: relative 18.1 %	~ 960	10 < C ≤ 100

M; males, F; females

In the 28 day dermal toxicity study (OECD TG 410) dose-dependent skin irritating effects have been observed at days 5-11, the effects were not apparent during the second week of dosing and no further changes were observed after that time point. The most pronounced effects were observed at day 6 at a dose level of 1000 mg/kg bw, in which 8 out of 10 animals were affected (slight (n=2), well defined (n=5) to moderate to severe skin reactions (n=1)) accompanied with desquamation (n=6).

Skin irritation effects have been also observed in a prenatal developmental toxicity study (OECD 414), in which pregnant rabbits were exposed to 0, 50, 200, 800 mg/kg bw/d (semi-occlusive exposure, 6 hours per day, no vehicle used) from GD 6-28. For the severity of damage the responses are evaluated according to the Draize score ranking from '0' ('no response') up to '4' ('severe response'). Most pronounced effects were observed on day 16 and on day 23 of application at the highest dose group (800 mg/kg bw/d). The erythema and oedema score at day 16 and 26 was 2.56 and 2.71 (incidence 100), and 2.66 and 2.81 (incidence 100), respectively. The erythema and oedema score at the dose level of 200 mg/kg bw/d was 1.9 and 1.64 at day 16, and 1.86 and 1.49 at day 23, indicating that observed effect does not worsen during the last week of exposure.

RAC agrees with the DS that the skin irritating effects observed in the repeated dose toxicity studies carried out with rats (28d study) and rabbits (prenatal developmental toxicity study) are considered dose dependent, however the effects were less severe or did not worsen at the end of the studies. The severity of the observed effects, which are pronounced at the highest dose levels (800 mg/kg bw/d (rabbit) to 1000 mg/kg bw/d (rat)) do not warrant classification for STOT RE effects.

Therefore, RAC concurs with the DS submitter that the adverse skin irritating effects observed are not severe enough.

RAC agrees with the DS that based on the observations described in the oral and dermal repeated dose toxicity studies **no classification for STOT RE is warranted**.

4.8 Germ cell mutagenicity (Mutagenicity)

4.8.1 Genotoxicity

4.8.1.1 In vitro

Table 23: Summary of in vitro tests

Method/ Guideline	Test system (Organism, strain)	Concentra-tions tested (give range)	Results		Remarks give information on cytotoxicity and other	Reference
			+ S9	- S9		
Bacterial reverse mutation test, OECD 471	*Salmonella typhimurium*: TA 98, TA 100, TA 102, TA 1535 and TA 1537	0-5000 µg/plate	Negative	Negative	No cytotoxicity Test substance: Margosa CO_2 extract; Batch: 420003	Uhde H, 2003 LPT Report no. 16320/02
Mammalian chromosome aberration test, OECD 473	Chinese hamster lung fibroblast V79 cells	0-5000 µg/mL	Positive (slightly, but stat. signifi-cant)	Negative	Cytotoxicity at 5000 µg/mL; slight increase of reciprocal translocations at this concentration (+ S9) Test substance: Margosa CO_2 extract; Batch: PM900201	Herold K, 2003 Kesla Report no. KBL/2003/1413 CHRt
Mammalian cell gene mutation test, OECD 476	Chinese hamster lung fibroblast V79 cells	0-5000 µg/mL	Negative	Negative	No cytotoxicity Test substance: Margosa CO_2 extract; Batch: PM900201	Herold K, 2003 Kesla Report no. KBL/2003/1413 HPRT

4.8.1.2 In vivo

Table 24: Summary of in vivo tests

Method/ Guideline	Species, Strain, Sex, No/group	Route and Frequency of application	Sampling times	Dose levels	Results give dose, sampling time and result +/-/+	Remarks	Reference
Mammalian erythrocyte micro-nucleus test, OECD 474	Mouse, NMRI, 5 M + 5 F	Oral, single dose	24, 48, h	0, 500, 1000, 2000 mg/kg bw	Negative	PCE/NCE ratio was unaffected. Test substance: Margosa CO_2 extract; Batch: 420003	Uhde H, 2003 LPT Report no. 16321/02

4.8.2 Non-human information

4.8.2.1 In vitro data

Margosa Extract was tested as neem oil in five strains of *Salmonella typhimurium* by reverse mutation assay (Ames-Test). No cytotoxicity, no increase in revertant colony numbers as indications for gene mutation was detected in any strain at concentrations up to 5000 µg/plate.

In Chinese hamster lung fibroblasts (V79 cells) a slightly increased incidence of structural chromosomal aberrations at the highest concentration of 5000 µL/mL in the presence of metabolic activation was detected. In a second experiment a slight increase in the aberration frequency was observed for the early sampling time only, i.e. this effect was not observed for the late sampling time. The changes observed were not dose-related, i.e. were only observed at the highest concentration tested, where cytotoxicity was observed. Nevertheless, the results with metabolic activation were regarded as positive due to statistical significance.

In a gene mutation test in V79 cells a significant increase in mutant frequency occurred at two experimental points at an intermediate concentration level (1.1 µL/mL) in the 1st experiment with metabolic activation and in the 2nd experiment without metabolic activation. Since these increases in either the presence or absence of metabolic activation occurred only in one of the two independent experiments (i.e., the effect was not reproducible) and due to the absence of concentration-relationship, the observed increases were considered coincidental and therefore regarded as negative. In conclusion, the HPRT test result was considered as non-mutagenic for *Margosa Extract*.

4.8.2.2 In vivo data

Margosa extract (tested as neem oil) was not genotoxic in the *in vivo* micronucleus test in mice exposed at dose levels up to and including 2000 mg/kg. At the two tested sampling times no increase of micronucleated polychromatic erythrocytes (PCE) was observed. The positive control cyclophosphamide induced significant increases in micronucleated PCEs.

4.8.3 Human information

No human information submitted by the applicant.

4.8.4 Other relevant information

No data submitted by the applicant.

4.8.5 Summary and discussion of mutagenicity

In conclusion, based on the results of *in vitro* and *in vivo* genotoxicity tests, including adequate positive and negative study controls, *Margosa Extract* can be evaluated to be unlikely to pose a genotoxic risk to humans.

4.8.6 Comparison with criteria

Following criteria for classification for gem cell mutagens are given in CLP regulation:

CLP regulation
The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1B is based on: — positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or — positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: — somatic cell mutagenicity tests *in vivo*, in mammals; or — other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

No human data are available for *Margosa Extract*, hence a classification in category 1A is not possible. Neither *in vivo* heritable germ cell mutagenicity tests nor positive results from *in vivo* somatic cell mutagenicity tests in mammals are available, hence a classification in Category 1B is not possible. *In vitro* studies (mutagenicity, clastogenicity) and the respective *in vivo* study showed overall a negative outcome, hence a classification in Category 2 is considered not necessary.

4.8.7 Conclusions on classification and labelling

No classification for mutagenicity is considered necessary, as the criteria laid down in the CLP regulation were not met.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In vitro data

Margosa CO_2-ext. was tested in different *in vitro* assays (reverse bacterial mutation assay, Mammalian chromosome aberration/ cell gene mutation test and erythrocyte MN test).

In five strains of *Salmonella typhimurium* (Ames Test) with or without metabolic activation *Margosa CO_2-ext.* did not induce mutations at concentrations up to 5000 µg/plate.

In Chinese hamster lung fibroblast cells (V79 cells) a slightly increased incidence of structural chromosomal aberrations at the highest concentration of 5000 µl/mL in the presence of metabolic activation was detected. In a second experiment a slight increase in the aberration frequency was observed for the early sampling time only but not at the late sampling time. The changes observed were not dose related, i.e. were only observed at the highest concentration tested, where cytotoxicity was observed. Nevertheless, the results with metabolic activation were regarded as positive due to statistical significance.

In a gene mutation assay in Chinese hamster V79 cells *in vitro* (V79/HPRT), a significant increase in mutant frequency occurred at two experimental points at an intermediate concentration level (1.1 µl/mL) in the 1st experiment with metabolic activation and in the 2nd experiment without metabolic activation. Since these increases in either the presence or absence of metabolic activation occurred only in one of the two independent experiments (i.e., the effect was not reproducible) and due to the absence of dose-response, the observed increases were considered coincidental and therefore regarded as negative. In conclusion, the HPRT test result was considered negative for *Margosa CO_2-ext*.

In vivo data

Margosa CO_2-ext. was tested in an *in vivo* mammalian erythrocyte micronucleus test (OECD TG 474). *Margosa CO_2-ext.* was not genotoxic in the *in vivo* micronucleus test in mice exposed at dose levels up to and including 2000 mg/kg. At the two tested sampling times no increase of micronucleated polychromatic erythrocytes (PCE) was observed. The positive control cyclophosphamide induced significant increases in micronucleated PCEs.

In conclusion, based on the results of *in vitro* and *in vivo* genotoxicity tests, including adequate positive and negative study controls, it is considered unlikely that *Margosa CO_2-ext.* poses a genotoxic risk to humans.

Comments received during public consultation

No comments received during public consultation.

Assessment and comparison with the classification criteria

There are no human data available for *Margosa CO_2-ext*.

A slight increased incidence of structural chromosomal aberration were detected in V97 lung fibroblast cells at the highest concentration (5000 µl/mL) in the presence of metabolic activation, however the changes are not considered dose dependent and occurred at concentrations were cytotoxicity was observed. The increased mutant frequency at intermediate concentration levels (1st experiment with and 2nd experiment without metabolic activation) were not reproducible and are considered coincidental. The *in vitro* tests were negative.

The *in vivo* mammalian micronucleus test did not indicate any genotoxic potential at dose levels up to 2000 mg/kg bw.

RAC concurs with the DS that **no classification for germ cell mutagenic effects is warranted.**

4.9 Carcinogenicity

Table 25: Summary of carcinogenicity studies

Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/group	Dose levels	Results Main effects/ Target organs/ Tumors	NO(A)EL ppm (mg/kg bw/d)	LO(A)EL ppm (mg/kg bw/d)	Remarks	Reference
No study submitted - Justification for Non-Submission accepted								

No chronic or carcinogenicity study has been submitted for *Margosa Extract*. The waiving of such a study is deemed acceptable in view of the lack of pertinent findings in genotoxicity tests and repeat dose studies (up to the limit dose). According to "Guidance on information requirements" – Guidance on regulation (EU) No 528/2012… "*The Long-term toxicity study (≥ 12 months) does not need to be conducted if:*

- *Long-term exposure can be excluded and no effects have been seen at the limit dose in the 90-day study, or*
- *A combined long-term repeated dose/carcinogenicity study (8.11.1) is undertaken"*

As no adverse effects were observed in the 90-day study in rats up to approx. 1000 mg/kg bw/day and long-term exposure is not expected according to the use scenarios submitted by the applicant, omission of carcinogenicity study is justified for the biocidal procedure.

No human information submitted by the applicant.

4.9.1 Conclusions on classification and labelling

Data lacking to allow a firm conclusion, therefore no classification is proposed.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No chronic or carcinogenicity study is available for *Margosa CO_2-ext.* No human information has been submitted by the applicant. Therefore no classification is proposed by the DS due to data lacking.

Comments received during public consultation

A comment has been submitted by MS. The author clarified that in the 90 day repeated dose toxicity study adverse effects at the highest dose (liver weight change) were detected and that the statement of the DS in the background document that no adverse effects have been observed is not appropriate. DS clarified the contradiction and agreed to MS comment.

Assessment and comparison with the classification criteria

RAC concurs with the DS that **classification for carcinogenicity is not warranted due to the absence of relevant data**.

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

Table 26: Summary of reproduction toxicity studies

Method/ Guideline	Route of exposure	Species, Strain, Sex, No/group	Dose levels	Critical effect Parental, Offspring (F1, F2)	NO(A)EL Parental toxicity	NO(A)EL reproductive toxicity	Remarks	Reference
No study submitted - Justification for Non-Submission accepted								

A two-generation study has not been submitted for Margosa Extract. The waiving of such a study is deemed acceptable for the biocidal procedure in view of the lack of genotoxicity and of pertinent findings on reproductive organs in repeat dose toxicity studies as well as the overall observed low toxicity in all tests conducted.

No human information submitted by the applicant.

4.10.2 Developmental toxicity

Table 27: Summary of teratogenicity studies

Method/ Guideline	Route of exposure, Duration	Species, Strain, No/group	Dose levels	Critical effects 1) dams 2) fetuses	NO(A)EL Maternal toxicity	NO(A)EL Teratogenicity Embryotoxicity	Remarks	Reference
OECD 414	Dermal, semi-occlusive, GD 6-28	Rabbit, NZW, 35 F	0, 50, 200, 800 mg/kg bw/d	1) Local irritation considered adverse ≥200 mg/kg bw; systemic: bw gain ↓ at 200 and 800 mg/kg bw, not considered adverse 2) None	Local: 50 mg/kg bw/d Systemic: 800 mg/kg bw/d	800 mg/kg bw/d	Test substance: *MARGOSA EXTRACT* Batch: 040515	Cicalese R, 2006 RTC Report No. 44800

4.10.2.1 Non-human information

After dermal application of *Margosa Extract* to pregnant rabbits, local skin irritation occurred in all treated groups and was considered adverse from 200 mg/kg bw onwards. In addition, a slight, dose-related tendency towards reduction of maternal body weight gain was observed. Net body weight loss (body weight at necropsy minus gravid uterus weight and minus body weight at Day 0) was observed in mid and high dose females. This did not attain statistical significance. The extent of reduced body weight gain is not considered biologically relevant and was not regarded as an adverse effect, because body weight at the end of treatment was only marginally affected.

No embryo- or foetotoxicity was apparent. Small foetuses in all groups, including the control, were found mostly in litters of larger size and it appears that the higher proportion of such litters, rather than the treatment, contributed to the slightly increased number of small foetuses in the high dose group. Thus, the maternal and the developmental NOAEL is 800 mg/kg bw/d.

A prenatal toxicity study in rodents has not been submitted. According to Regulation (EU) No 512/2012, a pre-natal developmental toxicity study shall be initially performed on one species. Developmental toxicity should be determined in rabbits by the oral route.

Whether the rat or the rabbit is the more sensitive species in developmental toxicity studies depends on the test substance, its toxicokinetics and mode of action and cannot be generalized. In the case of *Margosa Extract* it appears that adult rabbits are slightly more sensitive to the local effects of repeated dermal exposure. On the basis of the submitted data sensitivity towards systemic effects appears to be comparable between the rabbit and the rat. The waiving of the rodent study is deemed acceptable in view of the lack of developmental toxicity in rabbits and the overall low toxicity seen in all tests conducted. Furthermore, no adverse effects were observed in reproductive organs in repeat dose studies.

4.10.2.2 Human information

No human information submitted by the applicant.

4.10.3 Other relevant information

No data submitted by the applicant.

4.10.4 Comparison with criteria

Table 28 present the CLP criteria.

Adverse effects on development:

Table 28: CLP criteria regarding adverse effects on development

CLP criteria
Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

There are no appropriate epidemiological studies available on developmental effects in humans. Hence, classification with Category 1A according to the CLP regulation is not possible.

The prenatal developmental toxicity was investigated in rabbits complying with international test guidelines and GLP. In rabbits, no findings in offspring relevant for a possible classification for developmental effects were reported. In summary, neither classification in Category 1B (H360D) nor Category 2 (H361d) according to CLP criteria is considered appropriate.

No data are available to judge whether there are specific effects on or via lactation (H362).

4.10.5 Conclusions on classification and labelling

Reproductive toxicity concerning sexual function and fertility cannot be addressed due to the absence of data.

Regarding developmental toxicity, the data are considered conclusive but not sufficient to trigger classification for such effects.

Regarding effects on or via lactation, this classification cannot be assigned due to the absence of any data for adverse effects on or via lactation (no information of human evidence indicating a hazard to babies during the lactation period, no multigeneration study and no information concerning ADME).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Margosa CO_2-ext. was tested in a dermal prenatal developmental toxicity study (PNDT; OECD TG 414). No generation toxicity study has been conducted.

After dermal application of the test substance (dose levels: 0, 50, 200, 800 mg/kg bw/d) to pregnant rabbits, local skin irritation occurred in all dose groups and was considered adverse from 200 mg/kg bw/d onwards (see sections above).

Furthermore, a slight, dose related tendency towards reduction of maternal body weight gain was observed. Net body weight loss (body weight at necropsy minus gravid uterus weight and minus body weight at Day 0) was observed in mid and high dose females without statistical significance. Therefore the reduced body weight gain is not considered biologically relevant and is not regarded as an adverse effect.

No embryo- or foetotoxicity was apparent. Small foetuses in all groups, including the control, were found mostly in litters of larger size and it appears that the higher proportion of such litters, rather than the treatment, contributed to the slightly increased number of small foetuses in the high dose group. Thus, the maternal and the developmental NOAEL is 800 mg/kg bw/d.

A prenatal toxicity study in rodents has not been submitted. Whether the rat or the rabbit is the more sensitive species in developmental toxicity studies depends on the test substance, its toxicokinetics and mode of action and cannot be generalised. In the case of *Margosa CO_2-ext.* it appears that adult rabbits (pregnant rabbits) are slightly more sensitive to the local effects of repeated dermal exposure than rats. On the basis of the submitted data sensitivity towards systemic effects appears to be comparable between the rabbit and the rat. No adverse effects were observed in reproductive organs in repeat dose toxicity studies.

In the PNDT rabbit study, no findings in offspring relevant for a possible classification for developmental effects were reported. Overall, the data were considered conclusive by the DS but not sufficient to trigger classification for such effects.

Reproductive toxicity on sexual function and fertility cannot be addressed due to absence of data. No data are available to judge whether there are specific effects on or via lactation.

Comments received during public consultation

One MS commented that "anti-fertility (contraceptive and abortive)" effects of oils and extracts are reported in studies with various mammalian species including humans and if relevant the data should be more deeply described and discussed. The DS clarified that those results are not applicable to *Margosa CO_2-ext.*, since adverse effects are reported in particular for oral intake of large amounts of neem preparations with unknown compositions.

Assessment and comparison with the classification criteria

According to the CLP Regulation, reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, developmental toxicity in the offspring as well as effects on or via lactation.

RAC concurs with the DS that reproductive effects on sexual function and fertility cannot be addressed due to absence of data. The observed antifertility effects of oils and extracts in studies with various mammals species (including) humans cannot be considered for classification purpose. RAC agrees with DS's conclusion that no data are available to judge whether there are specific effects on or via lactation.

RAC agrees that there are no signs of developmental toxicity effects in the PNDT study with rabbits. The observed small changes of foetus weights can be regarded as not substance related. No further data have been submitted. The developmental toxicity cannot be concluded due to limited data available.

RAC supports the conclusion from the DS that classification for reproductive toxicity cannot be assessed due to the absence of suitable data for sexual function and fertility, development and lactation.

4.11 Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity

No studies were submitted that were conducted with *Margosa Extract*.

4.11.1.2 Immunotoxicity

No studies were submitted that were conducted with *Margosa Extract*.

4.11.1.3 Specific investigations: other studies

No studies were submitted that were conducted with *Margosa Extract*.

4.11.1.4 Human information

4.12 Medical Data

Table 29: Summary of medical data

Kind of study (e.g. case reports)	Examination methods, number of individuals examined	Results	References
Reports of medical surveillance in the production of Margosa Extract (NeemAzal) in India.	For a period of three years, monthly observations in up to 17 employees are recorded. Data on physical examinations (lung function tests, blood pressure, vision) and on subjective health observations. 2 resp. 4 records per year include blood chemistry resp. haematology parameters	No negative health effects are reported in the three years observation period.	Venkataram T.V (2001, 2002, 2003). Unpublished reports.
For completeness only. 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 presently evaluated *Margosa Extract* but were added for documentation that a research in the open literature was performed. Health risks can 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.			
Review of the open literature on neem products. Data from human and animal studies.	Not applicable	Clinical cases in indigenous medical use of neem leaves, fruit kernels and seed oil in Asia and Africa are reported. E.g. hepatotoxicity and nephrotoxicity from leaves, allergenicity of neem pollen, acute toxicity including encephalopathy from neem oil.	Boeke, S.J. et al. (2004). Safety evaluation of neem-derived pesticides. *J Ethnopharmacol. 94: 25-41*. published.

Table 30: Summary of poisoning incidents following oral ingestion of margosa oil

Kind of study (e.g. case reports)/Location	**Oral Dose / Active Substance**	**Number/Sex of individuals presented**	**Severity /Diagnosis**	**Outcome**	**References**
Case Report / India	150 ml / Margosa oil	35-year old woman	Serious / bilateral vision loss (Symptoms comparable to methanol toxicity)	Improved after medical treatment	Bhaskar et al. 2010
Case Report / Chennai, Tamil Nadu, India	250 ml (suicidal) / NeemAzal-T/S (pesticide)	35-year old women	Serious / neurological toxicity, drowsiness, low sensorium	Recovered after intensive care	Iyyadural et al. 2010
Case Report / Colombo; Sri Lanka	NR / Margosa oil	14-month old male infant	Serious / toxic encephalopathy (afebrile generalised tonic clonic seizure including hepatomegaly)	Recovered after intensive care	Senanayake et al. 2009
Case Report / Maharashtra, India	NR (accidental ingestion)	5-year old boy	Serious / Status Epilepticus, cardio-respiratory arrest	Partly recovered; neuro deficits	Donghade et al. 2008
Case Reports / Bangalore; Lucknow; India	NR / Margosa oil	46 / 37 boys; 9 girls; mean age: 4 weeks – 10 years	Serious / seizure, altered sensorium, vomiting (30%), difffusecerebral oedema in 34 cases	31 recovered / 6 fatal / 9 residual defects (e.g. cortical blindness)	James et al. 2006
Case Report / Sri Lanka	5 teaspoons / Margosa oil	7-year old girl	Serious / toxic encephalopathy (status epilepticus); hepatic encephalopathy, respiratory arrest	Recovered after intensive care	Sri Ranganathan et al. 2005
Two Case Reports / Singapore	Case 1: 5 mL / Margosa oil Case 2: “few drops” / Margosa oil	Case 1: 5-month old male infant Case 2: 3-month old female infant	Serious/ toxic encephalopathy (case 1: generalised tonic clonic seizure; case 2: generalised convulsions, shallow respiration)	Recovered after intensive care	Lai et al. 1990
Case Report / Thanjavur, Tamil Nadu, India	1000 mL/ Margosa leaf extract	24-year old woman	Serious / loss of consciousness, absence of reflexes, cardiac and respiratory arrest	Recovered after intensive care	Sivashanmugham et al. 1984
Case Reports / Egmore/Chennai; India	25 – 60 mL / Unrefined margosa oil	12 cases: 3x < 6 month 6x 6 month – 3 years 3x > 3 years	10 fatal / 2 serious / persistent generalised convulsions respiratory failure, Reye’s syndrome	10 deaths 2 NR (recovered?)	Sundaravalli et al. 1982
Case Reports / Malaysia	5 – 30 mL / Margosa oil	13 cases of infants and children; mean age: 10 months; range: 21 days to 4 years; 10 females, 3 males	Serious/ 2 fatal / toxic encephalopathy and Reye’s syndrome	10 recovered after intensive care; 2 fatal; 1 retarded development	Sinniah and Baskaran 1981

Kind of study (e.g. case reports)/Location	Oral Dose / Active Substance	Number/Sex of individuals presented	Severity /Diagnosis	Outcome	References
Case Reports / India (Chennai) and Malaysia; Conference on Margosa oil poisoning	Various / Margosa oil	55 children in Chennai; India	Serious / Fatal / syndrome of vomiting, drowsiness, metabolic acidosis, encephalopathy, Reye's syndrome	Chennai: 90 % mortality	Sinniah et al. 1981 *

NR: Not reported;
*: Cases reported by Sundaravalli et al. 1982 are assumed to be included in the report since the reports are from the same medical center.

Evaluation of the literature on neem demonstrates evidence of poisoning incidents and side-effects in the use of neem products with unknown composition. "Margosa Oil" or "Neem Oil" is used as a traditional medicine in Asia and Africa. Case reports describe severe intoxications in children predominantly following oral administration of "Margosa Oil" as a home remedy for the treatment of various diseases (e.g. common cold, deworming). Vomiting, drowsiness, convulsions, metabolic acidosis, and encephalopathy are among the reported signs of poisoning, autopsy of fatal cases revealed liver damage. According to some authors, the findings resemble those of Reye's syndrome (e.g. Sinniah and Baskaran 1981, Sinniah et al. 1981, Sundaravalli et al. 1982). Most of the cases of acute poisoning were reported from the use of unrefined and not standardised home remedies lacking any quality control and containing unknown quantities of toxic substances genuine to the seeds or other parts of the neem tree. In addition, contamination with aflatoxins and/or other harmful compounds may contribute to the toxic profile of the ingested home remedies (e.g. Sinniah and Baskaran 1981, Sinniah et al. 1981, Niemann 2002). One case of suicidal intake of the pesticide NeemAzal-T/S (Parry Agro Ltd, Chennai, India; 1 % azadirachtin, 51 % vegetable oil, 45 % tensides) was reported from a 35-year old woman without evidence of renal or hepatic complications. She recovered completely after intensive care without long-term sequelae (Yiiadural et al. 2010).

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).

Margosa Extract exerts no acute toxicity up to the limit dose of 2000 mg/kg bw in rats. In addition, no signs of toxicity were observed in repeated dose studies in rats (up to 90 days) and rabbits (treatment day 6-28) following oral (rats) and dermal (rats and rabbits) exposure. Hence, poisoning from *Margosa Extract* up to the limit dose of 2000 mg/kg bw is not to be expected. This is supported by medical observations of workers in the production of *Margosa Extract*. No adverse health effects were observed in the three-year observation period.

4.12.1 Summary and discussion

No relevant information on *Margosa Extract* was submitted.

4.12.2 Comparison with criteria

No data available to allow a comparison

4.12.3 Conclusions on classification and labelling

Data lacking.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Currently no harmonized classification exists for *Margosa Extract*. The effect studies that are relevant for classification are presented in the following.

5.1 Degradation

Table 31: Summary of relevant information on degradation

Method	Results	Remarks	Reference
OECD 301D	73.5% after 28 days	readily biodegradable fulfilling the 10-day window criterion	Dengler (2005a), Study Code 20051094/01-AACB
Based on OECD 111	Azadirachtin, half-lives at 12 °C: pH 5 = 1731.5 h pH 7 = 363.9 h pH 8 = 75.6 h	hydrolytic degradation, increasing with pH	Szeto, S.Y. and Wan, M.T, 1996.
Based on OECD 111	Nimbin, half-lives at 12 °C: pH 5 = 1480.9 h pH 7 = 1783.2 h pH 9 = 1994.7 h	low hydrolysis rate with inconsistent effect of pH	Bockholt, K. (2006), UCLGmbH, Study No. PR050/28
Based on OECD 111	Salannin, half-lives at 12 °C: pH 5 = 16577.5 h pH 7 = 22063.1 h pH 9 = 6649.1 h	very low hydrolysis rate, increasing in the acidic and alkaline range	Bockholt, K. (2006), UCLGmbH, Study No. PR050/28

5.1.1 Stability

The assessment of the abiotic degradation of *Margosa Extract* was conducted based on studies, which were conducted with the constituent limonoids azadirachtin, nimbin and salannin. Due to the test methodology, abiotic degradation processes like hydrolysis, photolysis or phototransformation can only be determined/estimated for a single constituent and not for the mixture in its entirety. Thus, the hydrolysis tests (see Table 32) have been performed with purified Azadirachtin, Nimbin and Salannin instead of *Margosa Extract*. Likewise, the modelling of the phototransformation in air was conducted with the information for the limonoids Azadirachtin, Nimbin and Salannin, because a modelling for the complex mixture *Margosa Extract* is not feasible.

Hydrolysis:

Table 32: Hydrolytic degradation

Method /Guideline	pH	Temperature [°C]	Initial TS concentration, C_0 [µg/mL]	Reaction rate constant, K_h [1/h x 10^{-3}]	Half-life, DT_{50} [h]	Coefficient of correlation, r_2	Reference
Azadirachtin							
Method based on basic principles of EC C.7 and OECD 111	7.0	25	19	2.46	282	0.9942	Szeto, S.Y. and Wan, M.T, 1996. RI = 2 No GLP-study Test material: Azadirachtin Sigma Aldrich (> 95% purity), no batch number available)
	7.0	30		5.58	124	0.9956	
	4.1	35		2.48	279	0.9954	
	4.5			2.29	303	0.9977	
	5.0			2.52	275	0.9960	
	5.5			3.02	230	0.9969	
	6.0			3.37	206	0.9946	
	6.2[1]			2.71	256	0.9983	
	6.6			4.75	146	0.9974	
	7.0			12.0	57.8	0.9983	
	7.3[1]			15.8	43.9	0.9973	
	7.5			22.5	30.8	0.9982	
	8.0			58.0	12.0	0.9934	
	8.0[1]			67.7	10.2	0.9980	
	8.1[1]			48.8	14.2	0.9982	
	7.0	40		19.7	35.2	0.9978	
	7.0	45		33.8	20.5	0.9985	
Nimbin							
EC C.7 and OECD 111	5	35	3	1.08	235.2	0.840826	Bockholt, K., UCLGmbH, Study No. PR050/28, 2006. RI = 2 Test material: Nimbin (96 % purity), batch number Nim 181297, Trifolio M GmbH
	7	35		1.31	283.2	0.962594	
	9	35		47.6	316.8	0.996200	
	5	50		1.48	489.6	0.997451	
	7	50		2.09	297.6	0.995403	
	9	50		148.5	100.8	0.997000	
Salannin							
EC C.7 and OECD 111	5	35	6	0.198	2632.8	0.999865	Bockholt, K., UCLGmbH, Study No. PR050/28, 2006. RI = 2 Test material: Salannin (96 % purity), batch number Sal 041297, Trifolio M GmbH
	7	35		0.199	3504.0	0.972047	
	9	35		0.658	1056.0	0.993830	
	5	50		1.55	542.6	0.880890	
	7	50		0.446	1514.4	0.962422	
	9	50		2.36	266.4	0.998420	

[1] Hydrolysis test was conducted with natural water.

The hydrolysis of azadirachtin was studied in several aqueous buffer solutions of pH 4.1 to 8.1 at 25 to 45 °C. In addition, hydrolysis of azadirachtin was studied in 4 natural waters (pH 6.2 to 8.1). The

hydrolytic stability of azadirachtin is strongly pH-dependent as indicated by a significant increase in the rate of degradation with increasing pH. The DT_{50} values for azadirachtin differ from 303 h (pH 4.5 and 35 °C) to 12.0 h (pH 8). The DT_{50} at pH 7 and 35 °C is 57.8 h. Based on this value the DT_{50} was recalculated using the Arrhenius equation to reflect standard outdoor conditions (12 °C and pH 7) with an result of DT_{50} = 363.9 h. Recalculated half-lives for pH 5 and 8 are displayed in Table 31. The results of the hydrolysis tests conducted with natural waters are consistent with the results of the hydrolysis tests in the aqueous buffer solution.

A hydrolysis test with nimbin and salannin was performed according to EC guideline C.7 and OECD 111 at pH 5, 7, and 9 in sterile buffer solutions (Bockholt, 2006). The DT_{50} values for nimbin at 35 °C vary from 235.2 h (pH 5) to 316.8 h (pH 9). DT_{50} values for salannin at 35 °C range from 3504 h (pH 7) to 1056 h (pH 9). The hydrolysis of nimbin as well as salannin is influenced by the pH: The effect of pH is inconsistent for nimbin, whereas for salannin an increase of the hydrolysis rate in the acidic and alkaline range is observed. Based on the DT_{50} values at pH 7 and 35 °C the DT_{50} values for nimbin and salannin were recalculated using the Arrhenius equation to reflect standard outdoor conditions (12 °C and pH 7). Resulting DT_{50} were 1783.2 h and 22063.1 h for nimbin and salannin, respectively. Recalculated half-lives for pH 5 and 9 are displayed in Table 31. Hydrolysis products are not detectable for the three limonoids due to the technical limitations with regard to radiolabelling of the test substance and synthesis of reference substances.

The susceptibility of the limonoids to hydrolysis at standard outdoor conditions (12 °C, pH = 7) decreases from azadirachtin (DT_{50} = 363.9 h) and nimbin (DT_{50} = 1783.2 h) to salannin (DT_{50} = 22063.2 h). Consequently, hydrolysis might contribute to the degradation of azadirachtin and nimbin under environmental conditions, whereas hydrolysis processes are negligible for salannin.

Photolysis in water:

According to OECD Guidline 316 phototransformation in water might be a relevant degradation pathway for substances which have sufficient light absorption (λ > 290 nm). As the UV/VIS absorption spectrum of *Margosa Extract*, shows no significant absorption above 290 nm (Bär, 2005) no photodegradability of *Margosa Extract* is expected. Thus, it is justified not to perform an experimental photolysis study.

Phototransformation in air:

Table 33: Phototransformation in air

Method /Guideline	**Compound**	**Time-dependent OH-radical concentration [OH radicals cm^{-3}]**	**Overall reaction rate constant k [$cm^3 \times molecule^{-1} \times s^{-1}$]**	**Half-life [h]**	**Reference**
AOPWIN v1.91, 2000, US-EPA	Azadirachtin	24-h average 5.0×10^5	227.03×10^{-12}	1.696	Fàbregas, 2005, RI = 1 No GLP-study, QSAR-Modelling based on the Smiles-code of the three limonoids. QSAR-modelling requires no test material.
	Nimbin		306.12×10^{-12}	1.258	
	Salannin		290.55×10^{-12}	1.325	

Degradation of organic compounds in the atmosphere is mainly based on the reaction with hydroxyl radical. The tropospheric half-lives of the three limonoids in *Margosa Extract* were estimated using the AOPWIN program (Fàbregas, 2005). The program (US-EPA, 2000, Version 1.91) is based on a

quantitative structure analysis developed by Atkinson. The calculation method sums up the reactivity of all structural elements towards OH radicals. Using a 24-hours day and a mean daily OH concentration in air of 5.0×10^5 radicals/cm³, half-lives in air of 1.26 h for nimbin, 1.33 h for salannin and 1.70 hours for azadirachtin were calculated.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No estimation of biodegradation was conducted.

5.1.2.2 Screening tests

Table 34: Ready biodegradability

Method/ Guideline	Test type[1]	Test para-meter	Inoculum			Additional substrate	Test substance conc.	Degradation		Test material	Reference
			Type	Concen-tration	Adap-tation			Incub. period	Degree [%]		
OECD 301 D	ready	oxygen consumption (BOD)	activated sludge	4.91×10^4 CFU/mL inoculum; 1.47×10^4 CFU/vessel	no	no	2 mg TS/L	28 days	73.5 %	CO_2-extract from cold pressed oil from Neem seed without shell, batch number 040515	Dengler (2005a), RI = 1

[1] Test on ready biodegradability according to OECD criteria

The ready biodegradability of *Margosa Extract* (0.2 % azadirachtin A+B), was determined in a Closed Bottle Test according to OECD Guideline 301 D and Directive 92/69/EEC using activated sludge as inoculum. In this test *Margosa Extract* was degraded to 73.5 % within 28 days. Therefore, *Margosa Extract* has to be classified as readily biodegradable, fulfilling the 10-day window criterion.

5.1.2.3 Simulation tests

The technical active substance *Margosa Extract* consists mainly of a complex mixture of fatty acids along with a small amount of related triterpenoids (salannin > nimbin > azadirachtin). Since it is not possible to synthesize *Margosa Extract* chemically, radiolabelling of the active substance is not feasible.

No lead substance was defined, as the triterpenoids, considered to be mainly responsible for the insecticidal effect, account for less than 2 % in total. Only for the assessment of the distribution of *Margosa Extract* in the environment the physico-chemical properties of salannin have been considered, which is the triterpenoid with the highest proportion in *Margosa Extract*.

Since data on ready biodegradability are available for *Margosa Extract*, and thus classification of the active substance *Margosa Extract* is based on these data, results from literature considering the degradation behaviour of Azadirachtin A and B in soil and water-sediment-systems were only be regarded as additional information and are not described in this report.

5.1.3 Summary and discussion of degradation

It has been shown, that *Margosa Extract* degraded to 73.5 % in 28 days in a test according to OECD 301 D and is consequently classified as readily biodegradable, fulfilling the 10-day window criterion. The limonoids azadirachtin and nimbin are susceptible to hydrolysis whereas hydrolysis processes are negligible for salannin. Hydrolytic half-lives are 363.9 h, 1783.2 h and 22063.1 h at pH 7 and 12 °C for azadirachtin, nimbin and salannin, respectively. Direct phototransformation in water is

irrelevant for *Margosa Extract* degradation. Likewise indirect phototransformation is insignificant due to the negligible volatilization of *Margosa Extract*.

Therefore, it is expected that *Margosa Extract* undergoes hydrolysis as well as biodegradation under natural conditions. Neither hydrolysis products nor metabolites of biodegradation have been detectable due to the technical limitations with regard to radiolabelling of the test substance and synthesis of reference substances.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 35: Adsorption/Desorption

Method /Guideline	Compound	Mean retention time [min]	Mean k' (capacity factor)	Mean logKoc	Koc [L/kg]	Reference
OECD TG 121	Azadirachtin	3.979	1.928	2.157	144	Bockholt, 2005 RI = 2 Test material: CO_2-extract from cold-pressed neemseed oil without shells, batch number 040515
	Nimbin	5.106	2.757	2.904	809	
	Salannin	5.795	3.264	3.243	1766	

The adsorption behaviour of the constituent limonoids in *Margosa Extract* was investigated using the HPLC method procedure according to the OECD Guideline 121 with UV-detection at 210 nm (Bocholt 2005). The capacity factors of azadirachtin, nimbin and salannin were generated from the chromatograms of Margosa Extract. Identification of the respective peaks was made with calibration solutions of the individual components. The log Koc values were estimated based on linear regression and amount to 2.157, 2.904 and 3.243 for azadirachtin, nimbin and salannin, respectively. The Koc values of the limonoids are 144, 809 and 1766 L/kg for azadirachtin, nimbin and salannin, respectively. According to the mobility classification by McCall et al. (1980) azadirachtin is high mobile, whereas for nimbin and salannin low mobility is predicted.

5.2.2 Volatilisation

Due to the very low vapour pressure of *Margosa Extract* (3.8×10^{-7} hPa at 20 °C) and the small Henry's Law constants of the constituent limonoids (4.406×10^{-23} atm m^3/mol, 5.714×10^{-12} atm m^3/mol and 2.073×10^{-10} atm m^3/mol for azadirachtin, nimbin and salannin, respectively) only negligible volatilization and transfer to the atmosphere is expected. Thus, long-range transport and accumulation in air of *Margosa Extract* is not expected.

5.2.3 Distribution modelling

No distribution studies were conducted in addition to the HPLC-method according to OECD Guideline 121.

5.3 Aquatic Bioaccumulation

Table 36: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference

QSAR Estimation (BCFBAF)	Azadirachtin: BCF_{fish} = 3.35 L/kg wwt	based on measured log K_{OW} = 1.3	Fàbregas 2006
QSAR Estimation (BCFBAF)	Nimbin: BCF_{fish} = 44.3 L/kg wwt	based on measured log K_{OW} = 3.0	Fàbregas 2006
QSAR Estimation (BCFBAF)	Salannin: BCF_{fish} = 94.69 L/kg wwt	based on measured log K_{OW} = 3.5	Fàbregas 2006

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The intrinsic potential for bioconcentration in aquatic organisms has been estimated for *Margosa Extract* on the basis of physical and chemical properties of its constituents. Measured log K_{ow} values for the limonoids azadirachtin, nimbin and salannin were presented in the dossier, which are ranging from 1.3 to 3.5. Values of log K_{OW} greater than or equal to 3 indicate that the substance may bioaccumulate. Surface tension of the whole active substance was determined, resulting in high surface activity with a surface tension of 35.3 mN/m at 20 °C. This is significantly below the trigger of 60 mN/m and *Margosa Extract* should therefore be considered as a surface active compound. As surface active molecules could have a potential for bioaccumulation, the testing of the bioaccumulation in an appropriate species of fish might be necessary.

On the basis of their measured log K_{OW}, BCF values were calculated for the limonoid compounds azadirachtin, nimbin and salannin, resulting in BCF values below 100 L/kg wet weight (see table above).

Although these limonoids are known to show biological activity, the initial assessment for the bioconcentration potential should also be performed and discussed on the basis of the whole extract.

The active substance mainly consists of fatty acids (oleic, stearic and linoeic acid), bound as glycerides, but also as free fatty acids. It can be both assumed that the surface activity of the active substance is solely based on these constituents and that the partition coefficient log K_{ow} of these substances would be significantly higher than those for the limonoids. In literature it was reported that surface tension of fatty acids and triglycerides was around 30 mN/m, not exceeding 35 mN/m (Chumpitaz *et al.* 1999). This explains the low surface tension of the whole extract representing the active substance.

The glycerides and fatty acids present in the active substance are identical to the endogenous compounds in the fatty acid cycle of higher organisms. Fatty acids are ubiquitous available in the environment and important naturally occurring biological molecules, found in all living organisms. They may be regarded as having fundamental roles (i.e. they are the building blocks of structurally important molecules in cellular membranes and also serve as sources of energy for biological systems). They can be metabolised via β-oxidation in animals and plants. This is quantitatively the most significant pathway for catabolism of fatty acids and results in the final products CO_2 and acetyl coenzyme A (acetyl-CoA) which as such is further metabolised to CO_2 and water. They are also known to be rapidly biodegradable. For these reasons, a potential for bioconcentration of these compounds can be assumed, but testing of their bioaccumulation would neither provide further knowledge nor biological relevance in this context. It can be concluded that the fats and fatty acids present in *Margosa Extract* do not raise a concern.

5.3.1.2 Measured bioaccumulation data

No measured data on bioaccumulation are available.

5.3.2 Summary and discussion of aquatic bioaccumulation

Based on physiological considerations, the bioaccumulation of glycerides and fatty acids can be considered as not relevant for the assessment of bioaccumulation. The calculated BCF_{fish} values of azadirachtin, nimbin and salannin are below 100 L/kg wet weight and thus do not pose a concern for bioaccumulation.

5.4 Aquatic toxicity

Margosa Extract is gained by CO_2-extraction from cold pressed oil from Neem tree (*Azadirachta indica* A. Juss.) seed without shells. It consists of a complex mixture of fatty acids mostly bond in glycerides and the related limonids azadirachtin, nimbin, and salannin. Due to the specific extraction procedure, the composition of *Margosa Extract* significantly differs from the substance described in the CLH report "Margosa, ext. (CAS No. 84696-25-3)" which has been already approved as biocidal active substance for PT18 (insecticides). Both extracts show different composition regarding to the proportions of the individual triterpeonids and fatty acids. In addition, the intended biological effects and therefore field of use significantly differ between both extracts. For these reasons, a read-across from "Margosa, ext." (acting as an insecticide) to *Margosa Extract* (acting as a repellent) cannot be performed.

No lead substance is defined and the effect assessment is mostly based on results for the whole extract. While it is not known which components mostly contribute to the intended efficacy as a repellent, it can also be deduced that mainly the limonoids should be regarded as relevant for (potential) adverse effects on non-target organisms in the environment: The limonoids from neem tree are known to act as antifeedant and growth disruptor toward insects. Therefore the accompanying chemical analysis of the effect studies is based on salannin as the limonoid with the highest proportion in *Margosa Extract*. This also applies to recalculations to mean measured concentrations, if required. In addition, the effect assessment was supported by the physico-chemical properties of salannin for applying the equilibrium partitioning method.

Table 37: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
OECD 203, EU C.1 *Oncorhynchus mykiss*, semi-static, mortality, 96 h	LC_{50} (96 h) = 11.2 mg/L (c.i.: 9.7 – 12.8 mg/L)	results based on mean measured concentrations	Stäbler (2005a)
OECD 202, EU C.2 *Daphnia magna*, semi-static, immobilization, 48 h	EC_{50} (48 h) > 128 mg/L	results based on mean measured concentrations	Stäbler (2005b)
OECD 201, EU C.3 *Desmodesmus subspicatus*, static, growth inhibition, 72 h	NOE_rC = 1.05 mg/L E_rC_{50} > 237 mg/L	results based on mean measured concentrations	Dengler (2005b)

5.4.1 Fish

One acute study with fish was provided for the test substance *Margosa Extract*. Further long-term studies are not available. The study was considered to be both valid and acceptable (reliability of 2) and considered as key study for fish. After 96 h and based on mean measured concentrations, a LC_{50} of 11.2 mg/L was calculated (95 % c.i.: 9.7 – 12.8 mg/L).

5.4.1.1 Short-term toxicity to fish

The acute toxicity of *Margosa Extract* to fish was tested with rainbow trout (*O. mykiss*) in a 96 hour semi-static study according to OECD Test Guideline 203 (Stäbler 2005a). Six concentrations between 6.25 and 65.5 mg/L (nominal) were tested. Acetone was used as solvent and vehicle for the test substance, corresponding to 0.1 mL/L test tank water, and showed no mortality in a solvent control. Three hours after pouring the test substance in the water, small droplets of test item were observed at any test concentration, also at the side wall of the test tanks at 25.6 mg/L and at higher concentrations. However, this did not affect concentrations of salannin and could possibly be contributed to the test substance's high content of glycerides and fatty acids. Monitoring of test substance concentration was performed for salannin every 24 h, along with the renewal of test media.

Based on salannin, mean measured concentration of the test substance was 76.4 % of nominal and therefore below 80 %. Based on this, the concentrations of *Margosa Extract* had to be recalculated and presented as mean measured concentrations. The test fulfils the further validity criteria set in the guideline.

Sublethal effects were observed between 16 – 65.5 mg/L, fish had difficulties with maintenance of equilibrium and fish upside down with loss of equilibrium were observed. According to the results of the test, the LC_{50} of the test item after 96 h was determined to be 14.6 mg/L (nominal, 95 % confidence interval 12.7 – 16.8 mg/L), equivalent to 11.2 mg/L mean measured concentration (95 % c.i.: 9.7 – 12.8 mg/L).

Table 38: Acute toxicity to fish

Method / Guideline	Species	Endpoint / Type of test	Exposure		Results [mg/L]			Remarks	Test material	Reference
			design	duration	LC_0	LC_{50}	LC_{100}			
OECD 203, C.1	*Oncorhynchus mykiss*	mortality	semi-static	96 h	7.64	11.2 (9.7 – 12.8)	19.6	results based on mean measured concentrations	100% *Margosa Extract* batch number 040515	Stäbler (2005a) RI = 2

5.4.1.2 Long-term toxicity to fish

No data available.

5.4.2 Aquatic invertebrates

One acute study with *Daphnia magna* was performed with *Margosa Extract*. The study was considered to be both valid and acceptable (reliability of 2) and considered as key study for invertebrates. For 48 h, an EC_{50} > 128 mg/L was calculated based on mean measured concentrations.

5.4.2.1 Short-term toxicity to aquatic invertebrates

The toxicity of *Margosa Extract* to invertebrates was tested in an acute study with *Daphnia magna* according to OECD Test Guideline 202 following a semi-static test design (Stäbler 2005b). Immobilisation of test animals was assessed and concentration of test substance on the basis of salannin monitored over 48 h. Six concentrations between 10 and 189 mg a.s./L (nominal) were tested. Acetone was used as solvent and vehicle for the test substance, corresponding to 0.5 mL/L test medium, and showed no mortality in a solvent control.

During the course of the study, no immobilised animals could be observed in all controls and all treatment levels. At all concentration levels oily agglomerates (emulsion drops) of the test item solution were observed on the water surface. At 105 mg/L one daphnid was caught in an oily drop, but was not determined to be immobilised by the test laboratory. However, this does not affect the outcome of the study and it can be concluded that the EC_{50} exceeds the highest tested concentration.

Monitoring of test substance concentration showed that concentration of salannin was 67.9 % of nominal concentration, therefore requiring recalculation of results to mean measured concentrations of test substance. The test fulfils the validity criteria set in the test guideline. Since no significant effects were observed up to the highest tested concentration, it can be concluded that EC_{50} > 128 mg/L (mean measured) after 48 h.

Table 39: Acute toxicity to invertebrates

Method / Guideline	Species	Endpoint / Type of test	Exposure		Results [mg/L]			Remarks	Test material	Reference
			design	duration	EC_0	EC_{50}	EC_{100}			
OECD 202, C.2	*Daphnia magna*	immobilisation	semi-static	48 h	128	> 128	> 128	results based on mean measured concentrations	100 % *Margosa Extract* batch number 040515	Stäbler (2005b) RI = 2

5.4.2.2 Long-term toxicity to aquatic invertebrates

No data available.

5.4.3 Algae and aquatic plants

One 72 h growth study with the green algae *Desmodesmus subspicatus* was performed with *Margosa Extract*. The study was considered to be both valid and acceptable (reliability of 2), covering both acute and long-term endpoints and considered as key study for algae. After 72 h, a NOEC of 1.05 mg/L and an EC_{50} > 237 mg/L was calculated based on growth rate and mean measured concentrations.

Effects on algae was tested on basis of the unicellular green algae *Desmodesmus subspicatus* in accordance to OECD Test Guideline 201 (1984), in addition considering the draft update from 2002 (Dengler 2005b). Five concentrations between nominally 10 and 400 mg/L *Margosa Extract* were tested, using acetone as vehicle. The solvent control did not show significant effects of the vehicle. Growth was evaluated over 72 h and results provided on the basis of growth rate and biomass. The stability of the test substance was monitored on the basis of salannin during the course of the study.

Concentrations of salannin were below 80 % of nominal at the end of the study (between 24.5 – 87.5 %) and therefore concentrations of *Margosa Extract* had to be recalculated to mean measured concentrations. The test fulfils the validity criteria at the time of performance of the test. However, further calculations showed that the test slightly missed the validity criteria of the recent version of the guideline (OECD TG 201 from 2006): The mean coefficient of variation for section-by-section specific growth rates is 36.76 %, exceeding the required ≤ 35 %. A further look at the results revealed that a single outlier in the second replicate at 24 h causes this exceedance of validity. This slight deviation is considered as acceptable, because at the time of the test the updated guideline was not available and since this deviation does not seem to affect effect evaluation results of the study and sufficient exponential growth was demonstrated. The study was considered as acceptable with a reliability of 2.

After 72 h and based on growth rate and mean-measured concentrations, a NOE_rC of 1.05 mg a.s./L was determined, corresponding to nominally 4.1 mg/L. The E_rC_{50} exceeded the highest concentration tested, 72 h E_rC_{50} > 237 mg a.s./L (mean measured), corresponding to nominally > 400 mg/L.

Table 40: Growth inhibition on algae

Method / Guideline	Species	Endpoint / Type of test	Exposure		Results [mg/L]			Remarks	Test material	Reference
			design	duration	NOE_rC	E_bC_{50}[1]	E_rC_{50}[2]			
OECD 201, C.3	*Desmodesmus subspicatus*	growth inhibition	static	72 h	1.05	n.d.	> 237	results based on mean measured concentrations	100% *Margosa Extract* batch number 040515	Dengler (2005b) RI = 2

[1] calculated from the area under the growth curve; [2] calculated from growth rate

5.4.4 Other aquatic organisms (including sediment)

No further data available.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Degradation (section 5.1): *Margosa Extract* is considered as **readily biodegradable, fulfilling the 10-days window criterion**. Therefore, rapid degradation can be concluded.

Hydrolysis (section 5.1): Hydrolysis cannot be considered as relevant for *Margosa Extract*. According to the "Guidance on the application of the CLP criteria" hydrolysis might be considered for classification only when the longest half-life determined with the pH-range 4-9 is shorter than 16 days. Because the half-life for some of the constituents of *Margosa Extract* exceeds 16 days, hydrolysis will not be considered to demonstrate that the substance is rapidly degradable.

Adsorption/desorption (section 5.2): Not relevant for classification and labelling.

Volatilisation (section 5.2): Not relevant for classification and labelling. According "Guidance on the application of the CLP criteria", volatilization only represents removal of a chemical from the water phase, and not degradation. Therefore, Henry's Law constant cannot be used for assessment.

Mobility (section 5.2): Not relevant for classification and labelling.

Aquatic bioaccumulation (section 5.3): No BCF_{fish} based on testing data is available. However, log K_{OW} is < 4 for the limonoids, considered as relevant components for bioaccumulation of *Margosa Extract*. Therefore, a low bioaccumulation potential can be concluded.

Aquatic toxicity (section 5.4): No acute toxicity (EC_{50}/LC_{50} > 1 mg/L) was found; therefore *Margosa Extract* is considered as not acutely toxic to aquatic life. Based on data on growth inhibition to algae (NOE_rC > 1 mg/L) and the substance's rapid degradation, no toxicity to aquatic life with long lasting effects is expected.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Acute (short-term) aquatic hazard: *Margosa Extract* does not exceed the effect trigger for acute category 1 with $EC_{50} \leq 1$ mg/L. The lowest acute value is the 96h-LC_{50} of 11 mg/L from an acute toxicity test with rainbow trout.

Long-term aquatic hazard, NOEC-based system: Only a long-term toxicity study on algae with *Margosa Extract* is available providing a NOE_rC of 1.05 mg/L. The substance is considered as rapidly degradable. Therefore, no chronic classification is required.

Long-term aquatic hazard, surrogate system: Based on the substance's acute toxicity EC_{50}/LC_{50} > 10 mg/L and its rapid degradation and its log K_{OW} < 4, no chronic classification is required.

According to CLP-Regulation no classification with regard to the environment is required. Furthermore, no M-factors are required.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS initially proposed no classification as hazardous to the aquatic environment. However, following the public consultation, the DS changed their position to consider *Margosa CO_2-ext.* as not rapidly degradable which, in combination with aquatic toxicity tests, results in a classification proposal as Aquatic Chronic 3 – H412.

Degradation

Three hydrolysis studies were run on the purified limonoid constituents azadirachtin, nimbin and salannin at different pH and temperature. The susceptibility of the limonoids to hydrolysis under standard outdoor conditions (12°C, pH = 7) decreases from azadirachtin (DT_{50} = 363.9 h) and nimbin (DT_{50} = 1783.2 h) to salannin (DT_{50} = 22063.2 h). The hydrolysis of azadirachtin, performed with a method based on basic principles of OECD TG 111 and EC C.7, is pH-dependent as indicated by a significant increase in the rate of degradation with increasing pH (recalculated half-lives at 12°C: 1731 h at pH=5; 364 h at pH=7; 76 h at pH=8). Also the hydrolysis of nimbin and salannin, both carried out according to OECD TG 111 and EC C.7, are influenced by pH (for nimbin recalculated half-lives at 12°C: 1481 h at pH=5, 1783 h at pH=7, 1995 h at pH=9; for salannin recalculated half-lives at 12°C: 16577 h at pH=5, 22063 h at pH=7, 6649 h at pH=9). The DS concludes that hydrolysis might contribute to the degradation of azadirachtin and nimbin under environmental conditions, whereas hydrolysis processes are negligible for salannin.

No photodegradation study in water was performed since the UV/VIS absorption spectrum of *Margosa CO_2-ext.* shows no *significant* absorption above 290 nm.

The ready biodegradability of *Margosa CO_2-ext.* was determined in a Closed Bottle Test according to OECD TG 301D and Directive 92/69/EEC using activated sludge as inoculum. In this test *Margosa CO_2-ext.* was degraded to 60.5% within 7 days and 73.5 % after 28 days. In summary, the DS considers *Margosa CO_2-ext.* as readily biodegradable, fulfilling

the 10-day window criterion. It is therefore considered to be rapidly degradable for classification purposes.

In contrast to the initial assessment above resulting in no proposal for classification as hazardous to the aquatic environment, the DS in response to the comments received during PC (see below) revised their conclusion on the environmental classification. As a consequence and despite the total content of limonoids determined to be only 2.7 ± 0.4% w/w, the DS considered *Margosa CO_2-ext.* as not rapidly degradable for classification purposes based on the results of a ready biodegradability test (according to OECD TG 301F), conducted with the constituent azadirachtin demonstrating only 21.6% mineralisation in 28 days.

Bioaccumulation

No measured data on bioaccumulation is available but estimated BCF values calculated for the limonoid constituents azadirachtin, nimbin and salannin are included in the CLH report.

The bioconcentration factors in aquatic organisms (fish) were calculated using the BCFWIN Programme v2.15, 2000 by US_EPA. This QSAR estimation was conducted on the basis of measured log Kow values of the three limonoid constituents, ranging from 1.3 to 3.5. The obtained BCF-values (see table below) indicate a low potential of the three constituents of *Margosa CO_2-ext.* in aquatic organisms. All the resulting estimated BCF values were below 100 L/Kg wet weight and the DS concludes no concern for bioaccumulation.

Table: BCF-values for three constituents of Margosa CO2-ext.

Method	Results	Remarks	Reference
QSAR estimation (BCFBAF) BCFWIN Programme v2.15, 2000 by US_EPA	BCF_{fish}= 3.35 L/Kg wwt Azadirachtin	Based on measured log Kow = 1.3	Fàbregas, 2006
QSAR estimation (BCFBAF) BCFWIN Programme v2.15, 2000 by US_EPA	BCF_{fish}= 44.3 L/Kg wwt Nimbin	Based on measured log Kow = 3.0	Fàbregas, 2006
QSAR estimation (BCFBAF) BCFWIN Programme v2.15, 2000 by US_EPA	BCF_{fish}=94.69 L/Kg wwt Salannin	Based on measured log Kow = 3.5	Fàbregas, 2006

Ecotoxicity

Short-term aquatic toxicity data are available for all three trophic levels, with long-term toxicity data only available for algae. A summary of the relevant information is provided in the following table (the key endpoints used in hazard classification are highlighted in bold). All studies were performed under (semi-)static conditions with results expressed in terms of mean measured concentrations (mmc).

Table: Summary of relevant information on aquatic toxicity

Method	Test organism	Endpoint	Toxicity values in mg a.s./L	Reference
OECD TG 203 (EU C.1) semi-static	*Oncorhynchus mykiss* (Rainbow trout)	96-h LC_{50} (mortality)	**11.2** (c.i.: 9.7 – 12.8 mg/L)	Anonymous, 2005a
OECD TG 202 (EU C.2) semi-static	*Daphnia magna*	48-h EC_{50} (immobilisation)	> 128	Stäbler, 2005b
OECD TG 201 (EU C.3) static	*Desmodesmus subspicatus*	72-h ErC_{50} 72-h NOErC (growth inhibition)	> 237 1.05	Dengler, 2005b

No lead substance is defined for this botanical extract and the effects assessment is mainly based on results for the whole extract. While it is not known which constituents mainly contribute to the intended efficacy as a repellent, it can also be deduced that mainly the limonoids should be regarded as relevant for (potential) adverse effects on non-target organisms in the environment: The limonoids from the neem tree are known to act as anti-feeding and growth disruptor toward insects. Therefore the accompanying chemical analysis in the available effect studies is based on salannin as the limonoid with the highest proportion in *Margosa CO_2-ext.*. This also applies to recalculations to mean measured concentrations, where required.

One valid and reliable short-term toxicity study with rainbow trout (*O. mykiss*) is provided for *Margosa CO_2-ext.* in a 96-h semi-static test according to OECD TG 203. Six concentrations between 6.25 and 65.5 mg/L (nominal) were tested. Based on salannin, mean measured concentrations of the test substance were below 80 % of nominal. According to the results of the test, the LC_{50} of the test item (*Margosa CO_2-ext.)* after 96-h was determined to be 14.6 mg/L (nominal), equivalent to 11.2 mg/L mean measured concentration (95 % c.i.: 9.7 – 12.8 mg/L).

One valid and reliable short-term toxicity study with aquatic invertebrates is available for *Margosa extract* (100% purity of test material). A 48-h toxicity test was performed with *D. m*agna, according to OECD TG 202. Immobilisation was assessed at six concentrations tested between 10 and 189 mg a.s./L (nominal). The concentrations of salannin were 67.9 % of nominal values and were therefore recalculated to mean measured concentrations of the test substance. According to the test results, the 48-h EC_{50} was determined to be > 189 mg/L (nominal) equivalent to 128 mg/L mean measured concentrations.

One 72-h growth inhibition study with the green algae *D. subspicatus* was performed with *Margosa CO_2-ext.* The study, conducted in accordance with OECD TG 201 (1984 and 2002), covers both acute and long-term endpoints. Concentrations of salannin were below 80 % of nominal at the end of the study (between 24.5 – 87.5 %) and therefore concentrations of *Margosa CO_2-ext.* had to be recalculated to mean measured concentrations. After 72-h,

a NOErC of 1.05 mg/L and an ErC_{50} > 237 mg/L was calculated based on growth rate and mean measured concentrations.

Based on the information above, the DS concluded not to classify *Margosa CO_2-ext.* as hazardous to the aquatic environment. In contrast to the initial assessment, the DS in response to the comments received during PC (see below) revised their conclusion on the environmental classification based on the results of a ready biodegradability test (according to OECD TG 301F), conducted with the constituent azadirachtin demonstrating only 21.6% mineralisation in 28 days, resulting in a proposed classification as Aquatic Chronic 3 – H412.

Comments received during public consultation

Two MSs provided public comments. One MS supported the initial proposal not to classify *Margosa CO_2-ext.* for the environment.

The other MS had specific comments and questions on the three following points:

- Further clarity on the total percentage of limonoids and individual key active constituents, because their concentrations could affect the overall environmental fate, toxicity and classification.
- Further clarification on the available information on the degradability of the key active constituents and the consequence on the conclusion on the rapid degradability of the substance according to CLP criteria.
- The applicability of the surrogate approach on the basis of the acute aquatic toxicity information for fish as the most acutely sensitive organism. In addition, consideration of available aquatic toxicity data from other Margosa extracts was suggested, such as Chironomid data.

Finally, the MS also mentioned the use of mixture classification calculations to consider the likely contribution of the individual active constituents to the overall extract chronic aquatic toxicity, despite the uncertainties on their composition and degradability (see also section "Additional key elements" in the background document to this opinion).

Additional key elements

During PC one MSCA suggested to consider additional aquatic toxicity information not included in the CLH report submitted for the substance at issue. In particular, data referring to chronic studies: a 28-days fish NOEC of 0.0047 mg azadirachtin A/L, a 21-days NOEC for *Daphnia* of 0.27 mg azadirachtin A/L, a 21-days NOEC for Daphnia of 0.27 mg azadirachtin A /L and a 28-days NOEC for *Chironomus o*f 0.0016 mg azadirachtin A/L, all based on a study on technical azadirachtin included in the EFSA conclusion on the pesticide peer review of the active substance azadirachtin (EFSA Journal 2011;9(3):1858).

Moreover, the DS further clarified that the above ecotoxicity studies were performed with a test substance called 'azadirachtin technical' but never with purified azadirachtin A (which is considered the main biological active constituent). Azadirachtin technical is defined as *"an extract from seed kernels of the tropical neem tree Azadirachta indica, with azadirachtin A regarded as lead substance"* and only the reported results from these studies were additionally recalculated based on the content of the lead substance azadirachtin A.

In the present CLH proposal only test data for the specific *Margosa CO_2-ext.* were included in the CLH report. This extract is a UVCB substance approved for the biocidal use as insect repellent (PT 19) and consequently should not be considered equivalent to other UVCBs extracted with water and further processed with organic solvents (as for PT 18), because of 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).

Regarding rapid degradation and in accordance with the decision taken at the BPC WG ENV in December 2016 (after the submission of the present CLH proposal), the PBT status of *Margosa CO_2-ext.* was considered as being potentially P.

Assessment and comparison with the classification criteria

Degradation

Based on the results of an OECD TG 301D test using *Margosa CO_2-ext.*, the substance was demonstrated to be readily biodegradable. However, the study does not allow to draw conclusions if and to what extent the constituents undergo degradation. Azadirachtin, one of the key active constituents of *Margosa CO_2-ext.*, is considered as not being readily biodegradable based on an OECD TG 301F test, a study that has been submitted within the biocidal approval process of Margosa ext. [from the kernels of *Azadirachta indica* extracted with water and further processed with organic solvents] for the use as insecticide (PT 18). For the other limonoids (nimbin and salannin), information on hydrolysis half-life values is available indicating that they are above the trigger of 16 days in the pH range 4-9.

According to the Guidance on the Application of the CLP criteria (version 4.1, June 2015), the biodegradation of a complex substance presents general interpretation problems where each constituent of the substance may behave differently. The guidance also states that a complex substance, such as UVCBs should be regarded as not rapidly degradable if the constituents that are notrapidly-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 by applying the CLP cut off-values to trigger the consideration for classification (CLP, Annex I.1.1.2.2).

RAC notes that further supplemental information available within the BPC WG ENV documents supported the RAC opinion conclusion to consider *Margosa CO_2-ext.* as not rapidly degradable for classification purposes. In particular, calculations for ready biodegradability using QSAR (BIOWIN v4.10) are available for the limonoids azadirachtin (A and B), nimbin and salannin resulting in not being readily biodegradable for the relevant constituents.

On the basis of azadirachtin not being readily biodegradable and lack of data for the other limonoids, RAC considers *Margosa CO_2-ext.* as not rapidly degradable for classification purposes.

Bioaccumulation

No measured BCF_{fish} data is available. The measured log K_{OW} for the limonoids is below the CLP trigger value of ≥ 4. Therefore, RAC agrees with the DS's conclusion that the substance has a low bioaccumulation potential.

Aquatic toxicity

Acute aquatic hazard

No acute toxicity below the CLP trigger value of L(E)C_{50} ≤ 1 mg/L was found. The lowest acute value is the 96-h LC_{50} of 11.2 mg/L (mmc) from an acute toxicity test with *O. mykiss.* Therefore *Margosa CO_2-ext.* does not fulfil the criteria and **no classification is proposed for acute aquatic hazards**.

Chronic aquatic hazard

With regard to chronic toxicity data, the NOErC = 1.05 mg/L for algae slightly exceeds the trigger for chronic hazard classification.

However, RAC agrees with the DS's revised assessment considering the substance as not rapidly degradable. As a consequence this **warrants classification as Aquatic Chronic 3 – H412** based on the fish 96-h LC_{50} of 11.2 mg/L (> 10 to ≤ 100 mg/L) for not rapidly degradable substances.

6 OTHER INFORMATION

No further data available.

7 REFERENCES

Author	Year	Title. Source (where different from company), Company, Report No., GLP (where relevant) / (Un)Published
Bär C.	2005	UV/VIS Absorption Spectrum, Infrared Absorption Spectrum, and ^{1}H-NMR Spectrum, of Margosa Extract: CO_2-Extract from cold pressed Oil from Neem Seeds without Shell, GAB GmbH, Report no.: 20051094/01-PCSD GLP: yes Published: no
Bhaskar M.V. et al.	2010	MR IMAGING FINDINGS OF NEEM OIL POISONING AJNR, 31, pp. E60 – E61
Boeke, S.J. et al.	2004	Safety evaluation of neem (*Azadirachta indica*) derived pesticides. J Ethnopharmacol. 94, pp. 25-41 Report no.: not applicable GLP: unknown Published: yes
Bockholt K.	2005	Estimation of the Adsorption Coefficient (Koc) on Soil and Sewage Sludge using High Performance Liquid Chormatography (HPLC) UCL GmbH Report no.: PR05/013 GLP: yes Published: no
Bockholt K.	2006	Hydrolysis as a Function of pH UCL GmbH Report no.: PR05/028 GLP: yes Published: no
Chevalier, F.	2003a	Acute Toxicity Study of Neemoil by Oral Administration to Rats. LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany Terra Nostra GmbH Report No 16315/02 GLP Unpublished
Chevalier, F.	2003b	Acute Toxicity Study of Neemoil in CD® Rats by Dermal Administration. LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany Terra Nostra GmbH Report No 16316/02 GLP Unpublished
Chevalier, F.	2003c	Acute Inhalation Toxicity Study of Neemoil in CD® Rats. LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany Terra Nostra GmbH Report No 16317/02 GLP Unpublished
Cicalese, R.	2005	Margosa Extract (CO2 extract from cold pressed neem seed oil) – 4 week dermal toxicity study in rat. RTC – Research Toxicology Centre, Roma, Italy Terra Nostra GmbH Report No 44070 GLP Unpublished
Cicalese, R.	2006a	Margosa Extract (CO2 extract from cold pressed neem seed oil) – 4 week dietary toxicity study in rats. RTC – Research Toxicology Centre, Roma, Italy Terra Nostra GmbH

Author	**Year**	**Title. Source (where different from company), Company, Report No., GLP (where relevant) / (Un)Published**
		Report No 43990 GLP Unpublished
Cicalese, R.	2006b	Margosa Extract (CO2 extract from cold pressed neem seed oil) – Prenatal developmental toxicity study in rabbits by dermal application. RTC – Research Toxicology Centre, Roma, Italy Terra Nostra GmbH Report No 44800 GLP Unpublished
Dengler D.	2005a	Assessment of the Ready Biodegradability of Margsoa Extract: CO_2-Extract from Cold Pressed Oil from Neem Seed without Shell GAB GmbH Report no.: 20051094/01-AACB GLP: yes Published: no
Dengler D.	2005b	Testing of Toxic Effects of Margosa Extract: CO_2-Extract from Cold Pressed Oil from Neem Seed without Shell to the Single Cell Green Alga Desmodesumus subspicatus GAB GmbH Report no.: 20051094/01-AADs GLP: yes Published: no
Dhongade R.K. et al.	2008	NEEM OIL POISONING. Indian Pediatrics, 45, pp. 56-57
Fàbregas E.	2005	Calculation of the Indirect Phototransformation Dr. Knoell GmbH Report no.: KC-PD-02/05 GLP: no Published: no
Fàbregas E.	2006	Calculation of the Bioconcentration Factor (BCF) Dr. Knoell GmbH Report no.: KC-BCF-01/06 GLP: no Published: no
Herold, K.	2003a	Chromosome Aberration Test in Chinese Hamster V79 Cells in vitro with Neem oil. Kesla Forschung & Service KG, Greppin, Germany Terra Nostra GmbH Report No KBL/2003/1413 CHRt GLP Unpublished
Herold, K.	2003b	Gene Mutation Test in Chinese Hamster V79 Cells in vitro with Neem oil. Kesla Forschung & Service KG, Greppin, Germany Terra Nostra GmbH Report No KBL/2003/1413 HPRT GLP Unpublished
Iyyadurai R. et. al.	2010	AZADIRACHTIN POISONING: A CASE REPORT. Clinical Toxicology, 48/8, pp. 857-858
James A, Meikandan D, Shankar SK, Mahadevan A, Behl HM,	2006	Neurotoxic effects of neem oil poisoning Annals of Neurology 60, Suppl. 10, S32 Published

Author	**Year**	**Title. Source (where different from company), Company, Report No., GLP (where relevant) / (Un)Published**
Meenakshi-Sundaram S		
Khan PK, Awasthy KS	2003	Cytogenetic toxicity of neem. Department of Zoology, Patna Women's College, Patna, India Food Chem Toxicol. 41(10): 1325-8 Non-GLP Published
Lai S.M. et al.	1990	MARGOSA OIL POISONING AS A CAUSE OF TOXIC ENCEPHALOPATHY. Singapore Med J., 31; pp. 463-465
Leuschner, J.	2003a	Acute Skin Irritation Test (Patch Test) of Neemoil in Rabbits. LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany Terra Nostra GmbH Report No 16318/02 GLP Unpublished
Leuschner, J.	2003b	Acute eye irritation study of Neemoil by instillation into the conjunctival sac of rabbits. LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany Terra Nostra GmbH Report No 16319/02 GLP Unpublished
Mahboob M, Siddiqui MK, Jamil K.	1998	The effect of subacute administration of a neem pesticide on rat metabolic enzymes. Toxicology Unit, Indian Institute of Chemical Technology, Hyderabad, India J Environ Sci Health B. 33(4): 425-38. Non-GLP Published
McCall Ph.J. *et al*.	1980	Measurement of Sorption Coefficients of Organic Chemicals and their Use in Environmental Fate Analysis. Protocols for Environmental Fate and Movement of Toxicants Symposium Proceedings, 94th annual meeting, 21-22, Washington DC GLP: no Published: yes
Meirelles A.J.A., Chumpitaz L.D.A., Coutinho L.F.	1999	Surface Tension of Fatty Acids and Triglycerides. JAOCS 76(3), 379-382 GLP: no Published: yes
Mishra RK, Singh SK	2005	Effect of aqueous leaf extract of *Azadirachta indica* on the reproductive organs in male mice. Department of Zoology, Banaras Hindu University, Varanasi, India. Indian J Exp Biol. 43(11): 1093-103. Non-GLP Published
Mitchell M.J., Smith S.L., Johnson S., Morgan E.D.	1997	Effects of the Neem Tree Compounds Azadirachtin, Salannin, Nimbin, and 6-Desacetylnimbin on Ecdysone 20-Monooxygenase Activity Archives of Insect Biochemistry and Physiology 35:199–209 (1997) GLP: no Published: yes
Mukherjee S, Garg S, Talwar GP	1999	Early post implantation contraceptive effects of a purified fraction of neem (*Azadirachta indica*) seeds, given orally in rats: possible mechanisms involved. National Institute of Immunology, and International Center for Genetic Engineering and Biotechnology, New Delhi, India J Ethnopharmacol. 67(3): 287-96.

Author	**Year**	**Title. Source (where different from company), Company, Report No., GLP (where relevant) / (Un)Published**
		Non-GLP Published
Niemann, L., Stinchcombe, S., Hilbig, B.,	2002	TOXICITY OF NEEM TO VERTEBRATES AND SIDE EFFECTS ON BENEFICIAL AND OTHER ECOLOGICALLY IMPORTANT NON-TARGETED ORGANISMS. TOXICITY TO MAMMALS INCLUDING HUMANS The Neem Tree (*Azadirachta indica* A Juss.) and Other Meliaceous Plants. (Ed. Schmutterer), Weinheim, Germany: VCH, 607-623 Report-no. GLP/GEP: no Published: yes
Ramesh, E.	2009	Margosa Extract (CO2 Extract from Cold Pressed Neem Seed Oil) 90-Day Dietary Toxicity Study in Rats Followed by a 4 Week Recovery Period. Terra Nostra GmbH Report No G5018 GLP Unpublished
Roop JK, Dhaliwal PK, Guraya SS.	2005	Extracts of *Azadirachta indica* and Melia azedarach seeds inhibit folliculogenesis in albino rats. Department of Zoology and Fisheries, Punjab Agricultural University, Ludhiana, Punjab, India Braz J Med Biol Res. 38(6):943-7 Non-GLP Published
Salvador, M.	2005	Margosa Extract (CO2 extract from cold pressed Neem Seed Oil) - Delayed dermal sensitisation study in Guinea Pigs (Magnusson and Kligman test). Research Toxicology Centre, Roma, Italy Terra Nostra GmbH Report No 49060 GLP Unpublished
Schmutterer, H. (ed.)	2002	The Neem tree. Neem foundation, Mumbai/India Report no.: not applicable GLP: unknown Published: yes
Senanayake M.P. et al.	2009	MARGOSA (KOHOMBA) OIL INDUCED TOXIC ENCEPHALOPATHY FOLLOWING HOME REMEDY FOR INTESTINAL WORMS. Ceylon Med. J., 54/4: p. 140
Sinniah, D. & Baskaran, G.	1981	MARGOSA OIL POISONING AS A CAUSE OF REYE'S SYNDROME. The Lancet, 1/8218, pp. 487-489
Sinniah, D. et al.	1981	MARGOSA OIL POISONING IN INDIA AND MALAYSIA Transactions of the Royal Society of Tropical Medicine and Hygiene. 75/6; pp 903-904
Sivashanmugham, R. et al.	1984	VENTRICULAR FIBRILLATION AND CARDIAC ARREST DUE TO NEEM LEAF POISONING J. Ass. Phys. India, 32; pp. 610-611
Sri Ranganathan, S. et al.	2005	Kohomba oil induced encephalopathy : lessons in prescribing traditional medicines. Sri Lanka Journal of Child Health, 34, Pp. 94-95
Stäbler D.	2005a	Acute Toxicity Testing of Margosa Extract: CO2-Extract from Cold Pressed Oil from Neem Seed without Shell in Rainbow Trout (Oncorhynchus mykiss) (Teleostei, Salmonella) GAB GmbH Report no.: 20051094/01-AAOm

Author	**Year**	**Title. Source (where different from company), Company, Report No., GLP (where relevant) / (Un)Published**
		GLP: yes Published: no
Stäbler D.	2005b	Assessment of Toxic Effects of Margosa Extract: CO2-Extract from Cold Pressed Oil from Neem Seed without Shell on Daphnia magna using the 48h Acute Immobilisation Test GAB GmbH Report no.: 20051094/01-AADm GLP: yes Published: no
Sundarvalli, N. et al.	1982	NEEM OIL POISONING. The Indian J Pediat. 49/3; pp. 357-359
Szeto S.Y., Wan M.T.	1996	Hydrolysis of Azadirachtin in Buffered and Natural Waters J. Agric. Food Chem 1996, 44, 1160-1163 GLP: no Published: yes
Uhde, H.	2003a	Mutagenicity Study of Neemoil in the Salmonella Typhimurium Reverse Mutation Assay (In Vitro). LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany Terra Nostra GmbH Report No 16320/02 GLP Unpublished
Uhde, H.	2003b	Micronucleus Test of Neemoil in Bone Marrow Cells of the NMRI mouse by Oral Administration. LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany Terra Nostra GmbH Report No 16321/02 GLP Unpublished
Venkataram, T.V.	2002	Employees Health Record 2001 not applicable Trifolio-M GmbH Report-no. not applicable GLP/GEP: no Published: no
Ventakaram, T.V.	2003	Employess Health Record 2002 not applicable Trifolio-M GmbH Report-no. not applicable GLP/GEP: no Published: no
Ventakaram, T.V.	2004	Employees Health Record 2003 not applicable Trifolio-M GmbH Report-no. 4826, not applicable GLP/GEP: no Published: no

Additional references

European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance azadirachtin. EFSA Journal 2011;9(3):1858. [76 pp.]. doi:10.2903/j.efsa.2011.1858. Available online: www.efsa.europa.eu/efsajournal.htm

Assessment Report, *Margosa Extract*, cold-pressed oil of *Azadirachta indica* seeds without shells extracted with super-critical carbon dioxide Product-type 19 (Repellents and attractants), 2017

Assessment Report, *Margosa Extract* Product-type 18 (Insecticides, Acaricides and Products to control other Arthropods), 2011

8 ANNEXES

Doc IIIA6 (Human health toxicological evaluation):

Confidential Annex