Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

Chrysanthemum cinerariaefolium, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

EC Number: 289-699-3
CAS Number: 89997-63-7

CLH-O-0000007334-76-01/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 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

8 June 2023

draft CLH Report format

REGULATION (EC) NO 1272/2008 (CLP REGULATION),

ANNEX VI, PART 2

Proposal for Harmonised Classification and Labelling for a biocidal active substance

CLH REPORT

Chrysanthemum cinerariaefolium, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, extract)

EC Number: 289-699-3

CAS Number: 89997-63-7

Index Number:

Contact details of dossier submitter: Ministerio de Sanidad, S. G. de Sanidad, D.G. Salud Pública, Calidad e Innovación, Subdirección General de Sanidad Ambiental y Salud Laboral, Paseo del Prado 18-20, E - 28071 Madrid

Version number: 05 Date: 28 February 2022

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CLH REPORT

SUMMARY

1. PRESENTATION OF THE ACTIVE SUBSTANCE

Two separate CARs were submitted in support of the active substances.

Pyrethrins were notified as an existing active substance, by Botanical Resources Australia Pty Ltd. (BRA), McLaughlin Gormley King Company (MGK) and SC Johnson & Son Inc., and a CAR was issued in July 2010, by RMS Spain.

A second CAR was issued in July 2010 by RMS Spain in support of the active substance *Chrysanthemum cinerariaefolium*, Extract which was notified as an existing active substance, by Kenya Pyrethrum Information Centre (KPIC).

It was subsequently decided that the substances were technically equivalent and that a combined CAR should be produced.

In accordance with the REACH guidance[1] the extraction method used to prepare the active substance was included in the substance name resulting in two active substances:

- *Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.)
- *Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with supercritical CO_2 (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.)

This CLH report has been prepared for the active substance, "*Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.)". Due to the length of the name the substance will be referred to by the names used in the study reports and in general overview sections as "*Chrysanthemum cinerariaefolium* extract from HCS".

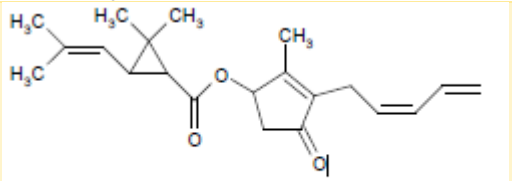
1.1 IDENTITY OF THE ACTIVE SUBSTANCE

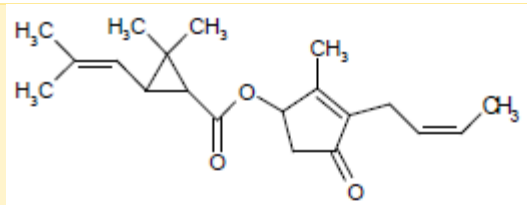
Table 1.1 Constituents

Constituent(s)	
ISO name	-
IUPAC or EC name	*Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.) Pyrethrin 1: (*Z*)-(*S*)-2-methyl-4-oxo-3-(penta-2,4-

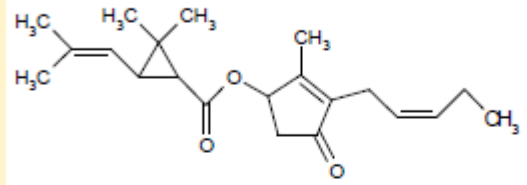
[1] Guidance for identification and naming of substances under REACH and CLP, Version 2.1 - May 2017.

	dienyl)cyclopent-2-enyl (1*R*)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate Cinerin 1: (*Z*)-(*S*)-3-(but-2-enyl)-2-methyl-4-oxocyclopent-2-enyl (1*R*)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate Jasmolin 1: (*Z*)-(*S*)-2-methyl-4-oxo-3-(pent-2-enyl)cyclopent-2-enyl (1*R*)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate Pyrethrin 2: (*Z*)-(*S*)-2-methyl-4-oxo-3-(penta-2,4-dienyl)cyclopent-2-enyl (*E*)-(1*R*)-trans-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropane carboxylate Cinerin 2: (*Z*)-(*S*)-3-(but-2-enyl)-2-methyl-4-oxocyclopent-2-enyl (E)-(1*R*)-trans -3-(2-methoxycarbonylprop-1-enyl)-2,2 dimethylcyclopropane carboxylate Jasmolin 2: (*Z*)-(*S*)-2-methyl-4-oxo-3-(pent-2-enyl)cyclopent-2-enyl (*E*)-(1*R*)-trans-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropane carboxylate
EC number	*Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.): 289-699-3 *Chrysanthemum cinerariaefolium*: 289-699-3 Pyrethrins: 232-319-8 Pyrethrin 1: 204-455-8 Pyrethrin 2: 204-462-6 Cinerin 1: 246-948-0 Cinerin 2: 204-454-2 Jasmolin 1: not available Jasmolin 2: not available
CAS number	*Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.): 89997-63-7 *Chrysanthemum cinerariaefolium*: 89997-63-7 Total Pyrethrins: 8003-34-7 Pyrethrin 1: 121-21-1 Pyrethrin 2: 121-29-9 Cinerin 1: 246-948-0 Cinerin 2: 121-20-0 Jasmolin 1: 4466-14-2

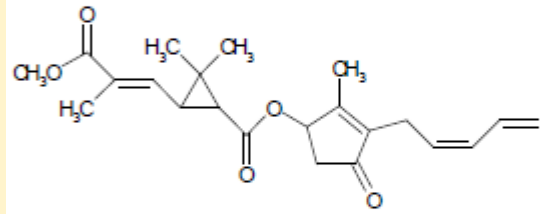
	Jasmolin 2: 1172-63-0
Index number in Annex VI of CLP	-
Minimum purity / content	100% w/w
Structural formula	 pyrethrin 1



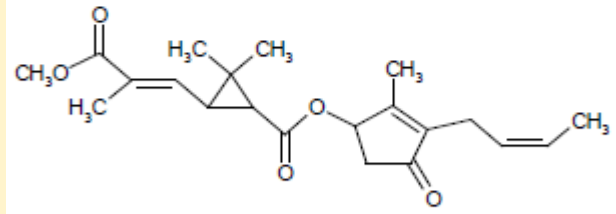
cinerin 1



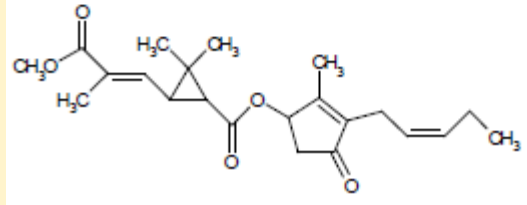
jasmolin 1



pyrethrin 2



cinerin 2



jasmolin 2

The active substance *Chrysanthemum cinerariaefolium* extract from HCS pure (synonym: Pyrethrum Extract) subject to the CLH proposal is an extract of the flower heads of *Chrysanthemum cinerariaefolium*. It contains Pyrethrins, which may be divided into the two groups Pyrethrins I (consisting of pyrethrin 1, cinerin 1, and jasmolin 1) and Pyrethrins II (consisting of pyrethrin 2, cinerin 2 and jasmolin 2). It also contains plant material, BHT and water.

Chrysanthemum cinerariaefolium extract from HCS has a minimum purity of 100% w/w

(UVCB substance). It is placed in the market as a solution, technical concentrate (c.a. 50% pyrethrins).

Table 1.2 Relevant impurities and additives

For information about the identity of impurities of the active substance, please refer to Confidential data in Appendix VI of this dossier (confidential information). For sake of completeness, information on the solvent used in all (eco)toxicology studies has been included in Appendix VI even though the active substance is stable without it and, therefore, the solvent should not be considered part of the active substance. This solvent (CAS 64742-47-8; EC 265-149-8) is present at a concentration q.s. 100% (solvent range 42.43-50.65%). The solvent has a harmonised classification as Asp. Tox. 1 (H304) and according to REACH registration dossier has no acute toxicity (oral LD_{50} > 5000 mg/kg bw, dermal LD_{50} > 2000 mg/kg/bw, and inhalation LC_{50} > 5.28 mg/L), skin sensitization (one key GPMT, nine supportive studies and three additional support data did not elicit a positive response) and aquatic toxicity properties that would influence the tests results or assessment of the hazard classes to be harmonised.

According to the "Hazard Classification and Labelling of Petroleum substances in the European Economic Area – 2020" from Concawe, for Kerosines, there is a chronic toxicity study on Daphnia magna which using WAF methodology gave a NOEL value of 0.48 mg/l based on reproduction (EMBSI, 2010), hence it would be considered as Aquatic Chronic 2. This is also supported by CLP inventory in ECHA website, with many Notified classifications including the classification as Aquatic Chronic 2 or 3.

Nevertheless, being the results from the ecotoxicity studies measured as total pyrethrins and being this solvent excluded from the reference a.s. definition, so its toxicity will not be used for classification purposes, and being the components pyrethrins I and II, total pyrethrins, much more toxic than the solvent and being classified as Aquatic Chronic 1 with a daphnia selected endpoint 0.00086 mg/L, we can conclude that the toxicity of this solvent will not interfere in the toxicity of the Chrysantemum extract.

The following terms are used throughout the CLH report:

• "Total pyrethrins" is a synonym to the active substance and the substance subject to CLH, i.e. it includes pyrethrins, plant material, BHT and water.

• "Extract" is the test substance, which includes in addition to total pyrethrins, also the solvent.

1.2 INTENDED USES AND EFFECTIVENESS

Table 1.3 Use of the active substance

Product type	MG03: Pest control PT18: Insecticides, acaricides and products to control other arthropods
Intended use pattern(s)	*Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents, is itended to be used as insecticide against a wide range of flying and crawling pests except those that are plant parasitic, in various applications sites in- and outdoor. Within this dossier the use against flies and mosquitoes is intended.
Users	Professionals and non-professionals

ES	*Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE ACCORDING TO THE CLP CRITERIA

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING FOR THE ACTIVE SUBSTANCE

Table 2.1 Proposed harmonised classification and labelling of the substance

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	*Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvent	289-699-3	89997-63-7	Acute Tox. 4 Acute Tox. 4 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H302 H332 H317 H400 H410	GHS07 GHS09 Wng	H302 H332 H317 H410		M=100 M=10 ATE oral = 700 mg/kg bw ATE inhalation = 2.5 mg/L (dusts and mists)	

A warning statement should be included in the summary of the product characteristics for the biocidal products containing *Chrysanthemum cinerariaefolium* extract from HCS indicating that the product contains an active substance which is dangerous or toxic to bees and that the product should only be applied in early morning or late evening when pollinators are unlikely to be foraging.

Table 2.2 Reason for not proposing harmonised classification and labelling and the status under CLH public consultation.

Hazard class	Reason for not proposing classification and labelling	Within the scope of public consultation
Explosives	data conclusive but not sufficient for classification;	Yes
Flammable gases (including chemically unstable gases)	hazard class not applicable	No
Oxidising gases	hazard class not applicable	No
Gases under pressure	hazard class not applicable	No
Flammable liquids	data conclusive but not sufficient for classification;	Yes
Flammable solids	hazard class not applicable	No
Self-reactive substances and mixtures	data conclusive but not sufficient for classification;	Yes
Pyrophoric liquids	data conclusive but not sufficient for classification;	Yes
Pyrophoric solids	hazard class not applicable	No
Self-heating substances and mixtures	hazard class not applicable	No
Substances which in contact with water emit flammable gases	data conclusive but not sufficient for classification;	Yes
Oxidising liquids	data conclusive but not sufficient for classification;	Yes
Oxidising solids	hazard class not applicable	No
Organic peroxides	data conclusive but not sufficient for classification;	Yes
Corrosive to metals	data conclusive but not sufficient for classification;	Yes
Acute toxicity via oral route	Acute Tox. 4, H302: Harmful if swallowed.	Yes
Acute toxicity via dermal route	data conclusive but not sufficient for classification;	Yes
Acute toxicity via inhalation route	Acute Tox. 4, H332: Harmful if inhaled.	Yes
Skin corrosion/irritation	data conclusive but not sufficient for classification;	Yes
Serious eye damage/eye irritation	data conclusive but not sufficient for classification;	Yes
Respiratory sensitisation	data lacking;	No

Skin sensitisation	Skin Sens. 1B, H317: May cause an allergic skin reaction.	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification;	Yes
Carcinogenicity	data conclusive but not sufficient for classification;	Yes
Reproductive toxicity	data conclusive but not sufficient for classification;	Yes
Specific target organ toxicity-single exposure	data conclusive but not sufficient for classification;	Yes
Specific target organ toxicity-repeated exposure	data conclusive but not sufficient for classification;	Yes
Aspiration hazard	data conclusive but not sufficient for classification;	Yes
Hazardous to the aquatic environment	Aquatic Acute 1, H400: M=100 Aquatic Chronic 1, H410: M=10	Yes
Hazardous to the ozone layer	data conclusive but not sufficient for classification;	Yes

NOTE eCA: Tests on physical hazards were conducted on the active substance as manufactured (TK, *ca*. 50% pyrethrins), that is, *Chrysanthemum cinerariaefolium* extract from HCS has been tested considering the solvent as part of the active substance. eCA Spain, ECHA and MSs have agreed that solvent should not be part of the active substance composition, therefore tests on physical hazards have been repeated on the purified active substance. However, *Chrysanthemum cinerariaefolium* extract from HCS does not meet any physical hazard class using the purified active substance. The details of the physical hazard tests conducted on the purified active substance (without the solvent) are described in section A.1.4 and A.1.5.

This plant extract has different components, which are classified:

Plant extract contains different components: pyrethrin 1, pyrethrin 2, cinerin 1, cinerin 2, jasmolin 1 and jasmolin 2. Pyrethrin 1, pyrethrin 2, cinerin 1 and cinerin 2 are included in Annex VI to CLP with the following classification:

Pyrethrin 1 – Index No. 613-023-00-1 – H302, H312, H332, H400, H410

Pyrethrin 2 – Index No. 613-024-00-7 – H302, H312, H332, H400, H410

Cinerin 1 – Index No. 613-025-00-2 – H302, H400, H410

Cinerin 2 – Index No. 613-026-00-8 – H302, H400, H410

Jasmolin 1 and Jasmolin 2 are not included in the C&L inventory, so self-classification is not available.

Other components of the extract (excluding the solvent), which are not included in Annex VI of CLP, have the following proposed classifications:

BHT – CAS 128-37-0 – H410 (REACH registration C&L)

Water – CAS 7732-18-5 – Not classified (Notified C&L)

Plant material:

Fatty acids:

Myristic acid – CAS 544-63-8 – Not classified (Notified C&L)

Palmitic acid – CAS 57-10-3 – Not classified (REACH registration C&L)

Stearic acid – CAS 57-11-4 – Not classified (REACH registration C&L)

Myristoleic acid – CAS 544-64-9 – Not classified (Notified C&L)

Palmitoleic acid – CAS 373-49-9 – H315, H319, H335 (Notified C&L)

Oleic acid – CAS 112-80-1 – Not classified (Notified C&L)

Linoleic acid – CAS 60-33-3 – Not classified (Notified C&L)

Terpenoids:

trans- β-farnesene – CAS 18794-84-8 – H304 (REACH registration C&L)

δ-cadinene CAS 483-76-1 – H315, H304 (Notified C&L)

trans-nerolidol - CAS 40716-66-3 – H400, H410 (REACH registration C&L)

Hexahydrofarnesyl acetone - CAS 502-69-2 – H400, H410 (REACH registration C&L)

Sesamin – CAS 607-80-7 – Not classified (Notified C&L)

β-sitosterol – CAS 83-46-5 – Not classified (Notified C&L)

α-amyrin – CAS 638-95-9 – H302 (Notified C&L)

β-amyrin – CAS 559-70-6 – H302 (Notified C&L)

Lupeol – CAS 545-47-1 – H302 (Notified C&L)

β-cubebene (CAS 13744-15-5), *cis*-Z-α-bisabolene epoxide (CAS not available), taxasterol (CAS 1059-14-9), pyrethrosin (CAS not available), γ-cadinene (CAS 39029-41-9), lupeyl acetate (CAS 1617-68-1), and aromadendrene (CAS 498-39-4) are not included in the C&L inventory, so self-classification is not available.

RAC general comment

This opinion refers to *Chrysanthemum cinerariaefolium* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with **hydrocarbon solvents**. In accordance with the REACH guidance, the extraction method used to prepare the active substance was included in the substance name resulting in two active substances:

1. *Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with **supercritical CO_2** (Redefined from Pyrethrins and Pyrethroids and Chrysanthemum cinerariaefolium, ext.), and
2. *Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with **hydrocarbon solvents** (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.).

Chrysanthemum cinerariaefolium, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with supercritical CO_2 is assessed in a separate RAC Opinion ((CLH-O-0000007335-74-01/F).

The active substance *Chrysanthemum cinerariaefolium* (EC number 289-699-3; CAS number 89997-63-7) extract from hydrocarbon solvents, is an UVCB substance, with a minimum purity of 100% w/w. It is a yellow liquid with no discernible odour, placed on

the market as a solution, technical concentrate, and intended to be used as an insecticide against a wide range of flying and crawling pests, in various applications sites in- and outdoors. The technical concentrate includes the presence of solvent which reduces the viscosity of the extract to make it easier to pour and mix during formulation, and to maintain the additive (butylhydroxytoluene, BHT) in solution. The active substance (which is subject to classification) contains pyrethrins, plant extract material (fatty acids, terpenoids and waxes), water, and BHT, which is added as a stabiliser to avoid oxidation of the pyrethrins.

Pyrethrins may be divided into the two groups: Pyrethrins I, consisting of pyrethrin 1, cinerin 1, and jasmolin 1, and Pyrethrins II, consisting of pyrethrin 2, cinerin 2 and jasmolin 2. According to the Applicant, the level of pyrethrins is adjusted using solvent to a nominal value of 50% of the sum of the above stated pyrethrins.

IUPAC or EC names, EC and/or CAS numbers, and harmonised classification, where available, of these substances are given in the table below:

IUPAC or EC name	**EC number** **CAS number**	**Harmonised classification**
Pyrethrin 1 (Z)-(S)-2-methyl-4-oxo-3-(penta-2,4-dienyl)cyclopent-2-enyl (1R)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate	204-455-8 121-21-1	Acute Tox. 4; H302 (Harmful if swallowed) Acute Tox. 4; H312 (Harmful in contact with skin) Acute Tox. 4; H332 (Harmful if inhaled) Aquatic Acute 1; H400 (Very toxic to aquatic life) Aquatic Chronic 1; H410 (Very toxic to aquatic life with long lasting effects)
Pyrethrin 2 (Z)-(S)-2-methyl-4-oxo-3-(penta-2,4-dienyl)cyclopent-2-enyl (E)-(1R)-trans-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropane carboxylate	204-462-6 121-29-9	Acute Tox. 4; H302 (Harmful if swallowed) Acute Tox. 4; H312 (Harmful in contact with skin) Acute Tox. 4; H332 (Harmful if inhaled) Aquatic Acute 1; H400 (Very toxic to aquatic life) Aquatic Chronic 1; H410 (Very toxic to aquatic life with long lasting effects)
Cinerin 1 (Z)-(S)-3-(but-2-enyl)-2-methyl-4-oxocyclopent-2-enyl (1R)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate	246-948-0 246-948-0	Acute Tox. 4, H302 (Harmful if swallowed) Aquatic Acute 1; H400 (Very toxic to aquatic life) Aquatic Chronic 1; H410 (Very toxic to aquatic life with long lasting effects)
Cinerin 2 (Z)-(S)-3-(but-2-enyl)-2-methyl-4-	204-454-2	Acute Tox. 4, H302 (Harmful if swallowed)

oxocyclopent-2-enyl (E)-(1R)-trans -3-(2-methoxycarbonylprop-1-enyl)-2,2 dimethylcyclopropane carboxylate	121-20-0	Aquatic Acute 1; H400 (Very toxic to aquatic life) Aquatic Chronic 1; H410 (Very toxic to aquatic life with long lasting effects)
Jasmolin 1 (Z)-(S)-2-methyl-4-oxo-3-(pent-2-enyl)cyclopent-2-enyl (1R)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate	not available 4466-14-2	Not included in the C&L inventory, so self-classification is not available
Jasmolin 2 (Z)-(S)-2-methyl-4-oxo-3-(pent-2-enyl)cyclopent-2-enyl (E)-(1R)-trans-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropane carboxylate	not available 1172-63-0	Not included in the C&L inventory, so self-classification is not available

Among these compounds, Pyrethrin 1 was selected as a surrogate for all the pyrethrins to generate the environmental fate data since it is representative of all other components, it is the component with the highest concentration (53%) and because it is difficult to evaluate the environmental fate properties of a mixture. The ecotoxicological studies were performed with the whole extract and the results based on nominal or measured concentrations of total pyrethrins (the six components in Pyrethrin I and II).

Other components of the extract (excluding the solvent) have the following proposed classifications:

BHT (CAS number 128-37-0) has a self-classification as Aquatic Chronic 1; H410 (Very toxic to aquatic life with long lasting effects) (REACH registration C&L).

Fatty acids in plant material:

- Myristic acid – CAS 544-63-8 – Not classified (Notified C&L)
- Palmitic acid – CAS 57-10-3 – Not classified (REACH registration C&L)
- Stearic acid – CAS 57-11-4 – Not classified (REACH registration C&L)
- Myristoleic acid – CAS 544-64-9 – Not classified (Notified C&L)
- Palmitoleic acid – CAS 373-49-9 – Skin Irrit. 2, H315; Eye Irrit. 2, H319; STOT SE 3, H335 (Notified C&L)
- Oleic acid – CAS 112-80-1 – Not classified (Notified C&L)
- Linoleic acid – CAS 60-33-3 – Not classified (Notified C&L)

Terpenoids in plant material:

- Trans- β-farnesene – CAS 18794-84-8 – Asp. Tox. 1, H304 (REACH registration C&L)
- δ-cadinene CAS 483-76-1 – Skin Irrit. 2, H315; Asp. Tox. 1, H304 (Notified C&L)
- Trans-nerolidol - CAS 40716-66-3 –Aquatic acute 1, H400; AChronic 1, H410 (REACH registration C&L)
- Hexahydrofarnesyl acetone - CAS 502-69-2 –Aquatic Acute 1, H400; Aquatic Chronic 1, H410 (REACH registration C&L)
- Sesamin – CAS 607-80-7 – Not classified (Notified C&L)
- Sitosterol Gamma – CAS 83-47-6– Not classified (Notified C&L)

- α-amyrin – CAS 638-95-9 – Acute Tox. 4, H302 (Notified C&L)
- β-amyrin – CAS 559-70-6 – Acute Tox. 4, H302 (Notified C&L)
- Lupeol – CAS 545-47-1 – Acute Tox. 4, H302 (Notified C&L)

Other substances in plant material that are not included in the C&L inventory and for which self-classification is not available include: β-cubebene (CAS 13744-15-5), cis-Z-α-bisabolene epoxide (CAS not available), taraxasterol (CAS 1059-14-9), pyrethrosin (CAS not available), lupeyl acetate (CAS 1617-68-1), and aromadendrene (CAS 498-39-4).

The following terms are used throughout the CLH report:

- "Total pyrethrins" is a synonym to the active substance and the substance subject to CLH, i.e. it includes pyrethrins (total pyrethrins: EC number 232-319-8, CAS number 8003-34-7), plant material, BHT and water.
- "Extract" is the test substance, which includes in addition to total pyrethrins, also the solvent.

RAC, however, notes that dose values in the CLH Report from the Biocides Competent Authority Report (CAR) stated as "Total pyrethrins", as well as the purity values, which were added from the Plant Protection Products Draft Assessment Report (DAR), represent those for the sum of pyrethrins I and II (without plant extract material, BHT, and water). In the DAR, the sum of pyrethrins I and II is named "Pyrethrins", "active ingredient" or "actual Pyrethrins" (as synonyms).

These dose levels have been recalculated by RAC, and in this RAC opinion, the term "total pyrethrins" represents the active substance as it is presently defined, i.e. sum of pyrethrins, plant extract material, BHT, and water. According to the latest reference specification document, the lowest percentage of "extract content" in the test substance (test substance = "extract content" plus solvent) is 60%. Therefore, RAC calculated the approximate "total pyrethrins" content with this percentage of "extract content" (60%), as the worst-case scenario[2].

For the environment, RAC agreed to use the endpoint values as such since the test substance contained in each case over 80% pyrethrins.

Although the active substance is stable without a solvent, and, therefore, the solvent should not be considered as part of the active substance, information on the solvent was presented by the Dossier Submitter. The solvent (distillates (petroleum), hydrotreated light; CAS 64742-47-8; EC 265-149-8)[3] is present at a concentration q.s. 100% (solvent range 42.43-50.65%). The solvent has a harmonised classification as Asp. Tox. 1 (H304) and according to the self-classification in the REACH registration dossier, it has no acute toxicity, skin sensitisation, or aquatic toxicity properties that would influence the test results or classification of the substance under the scope of the present harmonised

[2] E.g., if it was stated in the DAR that animals received 10 mg/kg bw/day of Pyrethrins (Pyrethrin Extract purity of 57%), it was roughly calculated that they received 17.5 mg/kg bw/day Pyrethrin Extract (test substance), leading to 10.5 mg/kg bw/day of "total pyrethrins" (pyrethrins, plant extract material, BHT, and water):

10 mg /kg bw/day Pyrethrins / 0.57 = 17.5 mg/kg bw/day of Pyrethrin Extract (test substance)

17.5 mg/kg bw/day of Pyrethrin Extract x 60% = 10.5 mg/kg bw/day of "total pyrethrins" (pyrethrins, plant extract material, BHT, and water)

[3] A complex combination of hydrocarbons obtained by treating a petroleum fraction with hydrogen in the presence of a catalyst. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C16 and boiling in the range of approximately 150°C to 290°C (REACH Registration Dossier).

classification proposal.

For the majority of the (eco)toxicity studies, the FEK-99 blend was used. It is a composite sample of Pyrethrum blended extracts from three members of the Pyrethrins Joint Venture consortium, MGK, Kenya, and Rwanda. Although most studies were performed with the FEK-99 blend obtained with hydrocarbon solvents, and only a few with the FEK-99 blend obtained with supercritical CO_2, the Applicant stated that the technical equivalence assessment studies supported read-across between the two extraction methods.

2.2 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Not applicable.

2.3 DATA SOURCES

Please refer to Appendix V for the reference lists and to Appendix VII for the studies included in DAR not in CAR.

3. SUMMARY OF THE HUMAN HEALTH RISK ASSESSMENT

3.1 SUMMARY OF THE ASSESSMENT OF EFFECTS ON HUMAN HEALTH

Table 3.1 Summary of the assessment of effects on human health

Endpoint	Brief description
Toxicokinetics	^{14}C-Pyrethrin was administered orally to male and female Sprague-Dawley rats. In a preliminary study time peak blood level and blood half-life were determined. In three definitive experiments, absorption, distribution, excretion and metabolism were examined (Selim, 1995). The definitive studies were conducted using three groups of 5 rats/sex/group as follows: a single low-dose (Group 1: 10 mg/kg bw), a single high-dose (Group 2: 50 mg/kg bw for females and 100 mg/kg for males) and a repeated low-dose (Group 3: non-radiolabelled Pyrethrin 1 at 10 mg/kg bw for 14 days prior to administration of a single oral low-dose of ^{14}C-Pyrethrin 1 on day 15). Two additional groups of 5 rats/sex/group were dosed exclusively for the purposes of isolation and identification of metabolites. In this ^{14}C-Pyrethrin 1 was administered orally at a single oral low-dose (Group 4: 10 mg/kg bw) and at a single oral high dose (Group 5: 50 mg/kg bw for females and 100 mg/kg for males). All rats survived the studies and no signs of toxicity were observed. The preliminary study showed that less of the administered ^{14}C-Pyrethrin 1 was systemically absorbed by the male rats than by the female rats with peaks in blood after 5-6 and 6-8 hours, respectively. Male rats excrete the ^{14}C-Pyrethrin 1 derived radioactivity faster than females, since the elimination half-time in males and females was 5.3 and 6.7 hours, respectively. In all groups of the definitive studies most of the radioactivity was excreted in the urine and faeces during the first 72 hours following administration. The levels of radioactivity expired as ^{14} CO_2 were very low. Regardless of dosage regimen, males excrete a majority of the administered radioactivity in the faeces via the enterohepatic circulation while females excrete approximately equal amounts in the urine and faeces. Repeated administration of Pyrethrin 1 increased the rate of elimination of the ^{14}C-Pyrethrin 1-derived radioactivity from the body in both males and females thus suggesting that repeated dose of ^{14}C-Pyrethrin 1 results in an induction of the liver microsomal enzyme system. Analysis of tissues at 7 days showed that in all dosing regimens, concentrations of radioactivity more than twice that of whole blood were found in liver, ovaries, carcass and fat. The radioactive residues in the fat were consistently higher than the residues in all other tissues analysed. The elimination of substantial amounts of ^{14}C-Pyrethrin 1-derived radioactivity in the faeces over and extended length of time indicated that enterohepatic circulation played a role in the elimination of the compound and/or its metabolites from the body.
Acute toxicity	Pyrethrum Extract showed acute toxicity by oral and inhalation routes, with an oral LD50 in rats for females (the most sensitive sex) of 700 mg/kg bw total pyretyhrins (1073 mg/kg bw extract) (Acute

	Tox. 4 – H302), a dermal LD50 in rabbits for both sexes higher than 2000 mg/kg bw total pyrethrins (3064 mg/kg bw extract) (without classification for this route of exposure), and an inhalation LC50 in rats of 2.5 and 3.9 mg/L for females and males respectively (Acute Tox. 4 – H332).
Corrosion and irritation	The compound was minimally irritating to the skin and eyes not requiring classification.
Sensitisation	A study conducted using the Buehler method did not indicate that *Chrysanthemum cinerariaefolium* extract from HCS is sensitising, however, three LLNA tests indicate sensitisation and, therefore, *Chrysanthemum cinerariaefolium* extract from HCS has been classified as Skin sensitisation, Category 1B, H317: May cause an allergic skin reaction.
Repeated dose toxicity	In sub-chronic tests for toxicity in mice, rats and dogs, the lowest relevant NOAEL after oral administration were 47, 57 and 18 mg /kg bw/d total pyrethrins (72, 87 and 28 mg/kg bw/d extract), respectively. The liver was the main target and increased liver weight was frequently accompanied with increased activity of transaminases. In addition, anaemia was observed in rats and dogs. Dermal administration of pyrethrins at doses up to 300 mg/kg bw/d total pyrethrins (460 mg/kg bw/d extract) for 21 days caused no systemic toxicity in rabbits. In a 13 weeks study in rats exposed by inhalation, the NOAEL for systemic toxicity was 11 mg/m^3. The effects described in the above paragraph for the oral application was observed.
Genotoxicity	Pyrethrins are non-genotoxic according to the available *in vitro* and *in vivo* studies.
Carcinogenicity	In a two-year study of toxicity and carcinogenicity in rats and an 18-month study of carcinogenicity in mice, the NOAEL were 4.4 and 14 mg/kg bw/d total pyrethrins (6 and 22 mg/kg bw/d extract), respectively. Rats showed at the highest dose (130-173 mg pyrethrins/kg bw/d total pyrethrins (199-265 mg/kg bw/d extract)) an increased incidence of adenoma of the liver in females, follicular cell adenomas of thyroid in both sexes and increased numbers of keratoacanthomas in males. Mice exhibited discoloured liver at the highest dose (690-830 mg pyrethrins/kg/d total pyrethrins (1051-1278 mg/kg bw/d extract)) together with vacuolar fatty change in the liver. No oncogenic effects could be detected in mice. Mechanistic studies indicate that tumours found in rat are species specific and caused by induction of CYP enzymes and the subsequent alteration of the metabolism of thyroid hormones. Therefore, an epigenetic mechanism is induced, and a threshold concentration must be assumed.
Reproductive toxicity	Pyrethrins did not show toxic effects on reproduction in a two-generation study on rats at dietary doses equivalent to 360 mg actual pyrethrins/kg bw/d total pyrethrins (552 mg/kg bw/d extract). In teratogenicity studies, the NOAEL for maternal toxicity and for fetotoxicity in rats was 75 mg/kg bw/d total pyrethrins (115 mg/kg bw/d extract). The NOAEL values for reproduction and teratogenicity in rabbits were 25 and 250 mg/kg bw/d total pyrethrins (38 and 383

	mg/kg bw/d extract), respectively. The NOAEL for development of F1 and F2 in a two-generation study was 12 mg/kg bw/d total pyrethrins (18 mg/kg bw/d extract).
Neurotoxicity	In a study of neurotoxicity in rats given single oral doses, acute neurological disorders and behavioural effects were noted, with a NOAEL of 20 mg/kg bw total pyrethrins (31 mg/kg bw extract).
Immunotoxicity	-
Disruption of the endocrine system	The available data package for *Chrysanthemum cinerariaefolium* extract from HCS has been assessed according to the specified guidance. The data show no effects of *Chrysanthemum cinerariaefolium* extract from HCS on the estrogen, androgen or steroidogenesis modalities. Although some findings related to the thyroid are noted in the rat with chronic and/or high dose exposure, no adverse effects on the thyroid are observed in other species (mouse and dog). Further, based on the available mode of action data, it can be concluded that the thyroid response in the rat is mediated secondary to hepatic enzyme induction and general systemic effects and is not relevant to humans. According to ECHA/EFSA "Guidance for the identification of Endocrine disruptors": - The potential for E,A,S-mediated adversity is considered to have been sufficiently investigated. Overall, there is strong weight of evidence to indicate that pyrethrum flower extract does not affect the estrogen, androgen, steroidogenesis modalities. - The potential for T-mediated adversity is considered to have not been sufficiently investigated. Overall, there is not sufficient weight of evidence to indicate that pyrethrum flower extract affects the thyroid modality by a mode of action that is specific to the rat and, as such, it cannot be concluded there are no indications of endocrine adversity of relevance to humans. A MoA (mode of action) has been described in the ED assessment report regarding thyroid histopathological changes on rats. Due to the age of some of the studies, they do not include all of the endocrine endpoints that would be required according to modern guidelines. But due to BPR article 90 restrictions, additional assays cannot be asked for. Therefore, regarding the endocrine disruption properties, no conclusion can be drawn as insufficient data is available for the assessment.
Other effects	In the historical data of MGK's employees, no serious adverse reaction to Pyrethrins have been reported. A few minor cases of dermatitis were noted, however, the implication of Pyrethrins is not confirmed. In the manufacturing plant of BRA over the last 25 years of manufacturing, no serious health problems have been recorded in workers either. Minor skin irritations have been observed following the harvesting operation however they have disappeared after washing with clean water and at worst within 1-2 days. Once the harvested crop material has been extracted, no cases of any health issues from handling the extracted and refined products have been noted. A few cases such as dermatitis, pruritus, nausea, a stinging

	sensation in the nasal and upper pharyngeal mucosa, moderate shortness of breath, cough productive of white phlegm without haemoptysis, fatigue, headache, dizziness and sensitisation were reported among the general population. However, people had recovered after few hours or in one case after 2 years.

3.2 REFERENCE VALUES

Table 3.2 Reference values

	Study	NOAEL/ LOAEL	Overall assessment factor	Value
$AEL_{short-term}$	Neurotoxicity	20 mg/kg bw/d total pyrethrins (31 mg/kg bw/d extract) based on observed tremors	100	0.20 mg/kg/d total pyrethrins (0.31 mg/kg bw/d extract)
$AEL_{medium-term}$	1-year study in dog	14 mg/kg bw/d total pyrethrins (22 mg/kg bw/d extract) based on hepatic damages	100	0.14 mg/kg/d total pyrethrins (0.22 mg/kg bw/d extract)
$AEL_{long-term}$	2-year study in rats	4.37 mg/kg bw/d total pyrethrins (6 mg/kg bw/d extract)	100	0.04 mg/kg/d total pyrethrins (0.06 mg/kg bw/d extract)
ADI	2-year study in rats	4.37 mg/kg bw/d total pyrethrins (6 mg/kg bw/d extract)	100	0.04 mg/kg/d total pyrethrins (0.06 mg/kg bw/d extract)
ARfD	Neurotoxicity study	20 mg/kg bw/d total pyrethrins (31 mg/kg bw/d extract) based on observed tremors.	100	0.20 mg/kg/d total pyrethrins (0.31 mg/kg bw/d extract)

4. SUMMARY OF THE ENVIRONMENTAL RISK ASSESSMENT

4.1 FATE AND BEHAVIOUR IN THE ENVIRONMENT

The fate and distribution in the environment was derived from studies on pyrethrin 1, since pyrethrin 1 represents the predominant analogue and a typical member (or paradigm) for the pyrethrum family. Therefore, it was regarded as feasible to make extrapolations from pyrethrin 1 to the active substance (*Chrysanthemum cinerariaefolium* extract from HCS). Hence, it was also considered to be justified to model the fate of total pyrethrins in the environment based on characteristics of pyrethrin 1.

Fate and behavior in the environment based on the physico-chemical properties of the active:

Fate and behaviour in air

The data presented predicts that the active substance would degrade rapidly in air under daylight conditions. Moreover, considering the substance's Henry's Law Constant (7.83E-02 Pa/m^3/mol at 25ºC), vapour pressure (6.9E-05 Pa at 25ºC) and octanol water partition coefficient (Log Kow: 5.59), none of the active will be lost to air according to the estimation of the fate of chemicals in a wastewater treatment plant as outlined in the TGD which is based on the SimpleTreat model 4.0. Therefore, emissions to air during the waste disposal stage need not be further considered. This data is important for the indoor-intended use products which contain the active.

Furthermore, this substance is unstable in the atmosphere to react with OH radicals and O_3 of troposphere. Its half-life in this compartment was calculated to be 1.28 h in the presence of OH radicals and 17.13 min in the presence of O_3. No major metabolites (at >10% AR) were detected in air.

Therefore, it is expected that the concentrations of the active substance in air will be negligible for all the uses presented in this dossier.

Fate and behaviour in aquatic compartment (including sediment)

Chrysanthemum cinerariaefolium extract from HCS (represented by pyrethrin 1) is characterized in the aquatic compartment by the following:

- Having a low water solubility, 0.23 mg/L at 20ºC
- Being hydrolytically stable in water at 25ºC, in the pH-range from 5 to 7, and unstable to pH 9 with a DT_{50} value of 17 days; with chrysanthemic acid as the single major metabolite. The concentration of chrysanthemic acid (at pH 9) increased in proportion to the decrease of Pyrethrin 1, accounting for 61% of the applied radioactivity (AR) after 30 days (Selim, 1995).
- Not readily biodegradable under the test conditions assessed (CO_2 Evolution – Modified Sturm Test). According to the OECD 301B guideline, substances are readily biodegradable in the CO_2 evolution test if CO_2 production is equal to or greater than 60% of the theoretical value within 10 days of the level achieving 10%.
- Undergoing rapid primary degradation in aerobic natural waters. In the Hein et al. (2017) test, calculated SFO DT_{50} values for pyrethrin 1 ranged from 6.7-10.7 days (at 20 ± 2°C). The main degradation product was pyrethrolone which reached a maximum of 9.5% AR after 21 days and then decreased to 2.8% AR at the last sampling interval. Several non-identified fractions were detected but

these were minor and/or composed of several peaks. Mineralisation reached a maximum of 7%.

- Under the influence of natural sunlight Pyrethrin 1 undergoes degradative processes in an aqueous solution at pH 7. After the irradiation numerous products were formed. However, no compound was identified. Its photolytic half-life in water was estimated to be 11.8 h.
- Pyrethrin 1 disappears rapidly when applied to aerobic water/sediment systems, taken from the natural environment, as a consequence of its low water solubility and its high adsorption coefficient, which leads to a fast movement into the sediment. In the Robinson and Wisocky (1994) test, degradation in water seems to proceed initially by a combination of hydrolysis and oxidation to form chrysanthemic acid and several low level degradates. A clear pattern of build-up and decline emerged for chrysanthemic acid, resulting in a maximum occurrence in the water/sediment system of 21.2% of applied radioactivity on day 21. The minor degradates were detected at various intervals, none exceeding 5% of the initial concentration of pyrethrin 1 at any point. Mineralisation was a minor degradation pathway (4% CO_2 at day 30).

  In the Witte (2007) study, performed in aerobic water/sediment systems (sampled from a pond and a creek), pyrethrin 1 was observed to move rapidly from the water phase into the sediment phase combined with a steadily increasing mineralization to CO_2 (32.0 and 50.7% AR at the end of the test for pond and creek systems, respectively) and breakdown in both aquatic and sediment phases to the metabolite Chrysanthemic acid. A substantial portion of the applied radioactivity became bound to sediment in both test systems. The amounts of bound residues were 29.3 and 39.5 % AR at the end of the test for pond and creek systems, respectively. In the sediment phase, chrysanthemic acid moves back to the water phase as it has a much better solubility and a lower adsorption (compared to pyrethrin 1); hence it is mainly found in the water phase during the test. In the pond system, chrysanthemic acid was detected at a maximum of 48.8% AR (on day 14) and 26.6% AR (on day 2) in the water and sediment phases, respectively, before declining thereafter; and in the total pond water/sediment system, it was detected up to a maximum of 65.6% AR after 14 days. In the creek system, chrysanthemic acid was detected at a maximum of 56.2% AR (on day 1) and 16.6% AR (on day 14) in the water and sediment phases, respectively, before declining thereafter; and in the total water/sediment system, it was detected up to a maximum of 66.8 % AR after 14 days.
- Based on the Robinson and Wisocky (1994) and Witte (2007) tests performed in aerobic aquatic environments, a geometric mean DT_{50} of 5.27 days was derived at 20°C (i.e. 10 days normalized to 12ºC).

Fate and behaviour in soil

Pyrethrin 1 rapidly degradates in soil under aerobic conditions, with a geomean half-life of 3.3 days at 20ºC. The following five laboratory soil degradation studies were performed to determine the rate of degradation of the substance.

In the Robinson (1994) study, degradation was assessed in a sandy loam soil; a half-life of 2.5 days at 20ºC was determined. Degradation seems to proceed initially by a combination of hydrolysis and oxidation to form several low-level metabolites (none formed at >10% AR). Residues in soil are initially extracted, but extended degradation is accompanied by the formation of residues that are bound to soil humus fractions. Soil residues are ultimately bound to soil humus fractions and mineralized (converted to carbon dioxide).

Mineralization and formation of bound residues also occurred in the Hein (2017) study, after pyrethrin 1 was applied onto a loam soil. In this study, a half-life of 2.96 days at 20ºC was obtained and no significant metabolites (at >5 %) were detected.

In the studies performed by Fifi (2015a, 2015b and 2015c), pyrethrin 1 exhibited low persistence in the loam, loamy sand and sandy loam soils tested. Half-lives of 4.69, 3.9 and 3 days were determined at 20ºC.

Adsorption/desorption study characterizes Pyrethrin 1 as immobile compound in soil according to estimated Koc values in different kinds of soil. Thus it can be expected that pyrethrin 1, once incorporated into the soil, will bind tightly to the soil particles.

Fate and behavior in the environment based on the representative products' use:

The active substance is used as an insecticide in the following representative products: Product A and Product B. Product A is a non-ready to use (non-RTU) ultra-low volume (ULV) aerosol; designed to target flying insects. It is for use by professionals only, as a spot application in indoor areas that are continuously occupied such as hospital wards, residential nursing homes and prisons. Product B is an electric vaporizer mat that provides protection from mosquitoes for about 8 hours. The vaporizer mat is solely for use by non-professionals, in private housings, during night-time hours.

Releases to the environment, from the use of the products, may occur during the preparation (relevant to Product A only), application and cleaning steps. To assess the worst-case use of the products, wet-cleaning has been assessed only; therefore, the STP is considered as the main "receiving compartment". Subsequent receiving compartments in the environment are therefore outdoor air (atmosphere), surface water, sediment, agricultural soil and groundwater. Emissions to these environmental compartments result from the cumulative emission from the preparation, application and/or cleaning steps, carried out indoors, following treatment with the products.

Table 4.4 Summary table of compartments exposed and assessed

Summary table on compartments exposed and assessed		
Compartment	Exposed (Y/N)	Assessed (Y/N)
STP	Y	Y (quantitative)
Surface water/sediment	Y	Y (quantitative)
Soil	Y	Y (quantitative)
Groundwater	Y	Y (quantitative)
Air	Y	Y (qualitative)

As indicated in the previous section, chrysanthemic acid was detected as the only relevant metabolite (present at >10% AR) in the hydrolysis (Selim, 1995) and aerobic water/sediment tests performed (Robinson and Wisocky (1994); Witte (2007)). Since in the Witte (2007) study, the highest maximum formations were obtained, these were considered in the risk assessment as a worst-case approach and are detailed in the following table. In the other studies performed (in air and soil), no major metabolites were detected.

Table 4.5 Summary table on relevant metabolites/degradants

Summary table on relevant metabolites/ degradants		
Metabolite/ degradant/transformation or reaction product	Compartment	% Active Substance
Chrysanthemic acid	Pond water/sediment system Pond water system	65.6 48.8

	Pond sediment system	26.6
	Creek water/sediment system Creek water system Creek sediment system	66.8 56.2 16.6

Input parameters used for calculating the fate and distribution of the active substance in the environment are presented in the table below.

Table 4.6 Summary table of relevant physico-chemical and fate and behaviour parameter of the active substance

Summary table on relevant physico-chemical and fate and behaviour parameter of the active substance – Pyrethrin 1			
	Value	**Unit**	Remarks
Molecular weight	328.4	g/mol	-
Melting point	142.27	°C	-
Boiling point	400.8	°C	-
Vapour pressure (25°C)	6.9E-05	Pa	-
Water solubility (20°C)	0.23	mg/L	-
Log Octanol/water partition coefficient (Log Kow)	5.59	Log 10	Kow = 389045.14
Organic carbon/water partition coefficient (Koc)	34674	L/kg	Log Koc = 4.54
Henry's Law Constant (25°C)	7.83E-02	$Pa/m^3/mol$	
Bioconcentration, aquatic	500	L/kg_{wwt}	Whole body BCF
Biodegradability	Not biodegradable		
DT_{50} for hydrolysis	115	d (at 25ºC)	At pH 7
DT_{50} for photolysis in surface water	11.8	h	
DT_{50} for biodegradation in surface water	10.7	d (at 20ºC)	Using SFO kinetics DT_{50} values ranging from 6.7-10.7 days were obtained (Hein *et al*., 2017). The highest DT_{50} was used as a worst-case.
DT_{50} for biodegradation in sediment	10	d (at 12ºC)	5.27 d at 20ºC
DT_{50} for degradation in air	1.28	hr	
DT_{50} for biodegradation in soil	3.3	d (at 20ºC)	Geomean value from five different soil studies/half-lives.

Effects assessment

Predicted no effect concentrations (PNEC), used in the environmental risk assessment of the parent compound, are listed in the table below.

Since chrysanthemic acid is several orders of magnitude less toxic than the parent substance (Mantilacci, 2015a), it can be assumed that the PNEC for the metabolite is covered by the PNEC of the parent substance (ENV 3, ECHA TAB 2018). In this case, the PNEC values for freshwater and sediment are the only relevant values for the assessment of chrysanthemic acid – since the metabolite was identified in these compartments only at >10% AR.

Table 4.7 Summary table of calculated PNEC values

Summary table of calculated PNEC values	
Compartment	PNEC
STP	2.3E-02 mg/L
Freshwater	8.6E-05 mg/L
Sediment	83.5 µg/kg_{dw}, equivalent to 1.82E-02 mg/kg_{wwt}
Soil	9E-03 mg/kg_{dw}, equivalent to 7.94E-03 mg/kg_{wwt}
Oral (secondary poisoning)	2.93 mg/kg diet

A. Assessment of intrinsic properties and effects of the active substance

A.1. General substance information

A.1.1. Identity of the substance

Table A.1 Summary table on substance identity

Summary table on substance identity	
Common name (ISO name, synonyms)	*Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.) pyrethrin 1, pyrethrin 2, cinerin 1, cinerin 2, jasmolin 1, jasmolin 2
Chemical name (EC name, CA name, IUPAC name)	*Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.) Pyrethrin 1: (*Z*)-(*S*)-2-methyl-4-oxo-3-(penta-2,4-dienyl) cyclopent-2-enyl (1*R*)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate Cinerin 1: (*Z*)-(*S*)-3-(but-2-enyl)-2-methyl-4-oxocyclopent-2-enyl (1*R*)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate Jasmolin 1: (*Z*)-(*S*)-2-methyl-4-oxo-3-(pent-2-enyl) cyclopent-2-enyl (1*R*)-trans-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane carboxylate Pyrethrin 2: (*Z*)-(*S*)-2-methyl-4-oxo-3-(penta-2,4-dienyl) cyclopent-2-enyl (*E*)-(1*R*)-trans-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropane carboxylate Cinerin 2: (*Z*)-(*S*)-3-(but-2-enyl)-2-methyl-4-oxocyclopent-2-enyl (*E*)-(1*R*)-trans -3-(2-methoxycarbonylprop-1-enyl)-2,2 dimethylcyclopropane carboxylate Jasmolin 2: (*Z*)-(*S*)-2-methyl-4-oxo-3-(pent-2-enyl)cyclopent-2-enyl (*E*)-(1*R*)-trans-3-(2-

ES	*Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents
	methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropane carboxylate
EC number	*Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.): 289-699-3 *Chrysanthemum cinerariaefolium*: 289-699-3 Pyrethrins: 232-319-8 Pyrethrin 1: 204-455-8 Pyrethrin 2: 204-462-6 Cinerin 1: 246-948-0 Cinerin 2: 204-454-2 Jasmolin 1: not available Jasmolin 2: not available
CAS number	*Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (Redefined from Pyrethrins and Pyrethroids and *Chrysanthemum cinerariaefolium*, ext.): 89997-63-7 *Chrysanthemum cinerariaefolium*: 89997-63-7 Total Pyrethrins: 8003-34-7 Pyrethrin 1: 121-21-1 Pyrethrin 2: 121-29-9 Cinerin 1: 246-948-0 Cinerin 2: 121-20-0 Jasmolin 1: 4466-14-2 Jasmolin 2: 1172-63-0
other CAS numbers (e.g. deleted, related, preferred, alternate)	CIPAC: Pyrethrins: 32 EU Index: Pyrethrins: 613-022-00-6
Molecular formula	pyrethrin 1: $C_{21}H_{28}O_3$ cinerin 1: $C_{20}H_{28}O_3$ jasmolin 1: $C_{21}H_{30}O_3$ pyrethrin 2: $C_{22}H_{28}0_5$ cinerin 2: $C_{21}H_{28}O_5$ jasmolin 2: $C_{22}H_{30}O_5$

ES	*Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents
Molecular weight or molecular weight range	pyrethrin 1: 328.4 g/mol cinerin 1: 316.4 g/mol jasmolin 1: 330.4 g/mol pyrethrin 2: 372.4 g/mol cinerin 2: 360.4 g/mol jasmolin 2: 374.4 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	This information can be found in the Appendix VI: Confidential Information.
Degree of purity (%)	100% w/w

Conclusion on the identity of the active substance

Chrysanthemum cinerariaefolium extract from HCS*,* pure (synonym: Pyrethrum Extract) is an extract of the flower heads of *Chrysanthemum cinerariaefolium*. It contains Pyrethrins, which may be divided into the two groups: Pyrethrins I (consisting of pyrethrin 1, cinerin 1, and jasmolin 1) and Pyrethrins II (consisting of pyrethrin 2, cinerin 2 and jasmolin 2).

The technical concentrate (TK) includes the presence of solvent to reduce the viscosity of the extract to make it easier to pour and mix during formulation, and also helps to keep the additive in solution (additive is added to avoid oxidation of the pyrethrins). eCA Spain, ECHA and MSs agreed that the solvent should not be part of the active substance, since the stability study shows that *Chrysanthemum cinerariaefolium* extract from HCS remains stable without the solvent, showing only a slight decrease in stability, which is not enough to support the inclusion of the solvent in the composition of the active substance. This decision is based on the legal definition of "substance" established in article 3(2) of the BPR, which excludes any solvent that may be separated from the substance without affecting the stability or changing its composition. Therefore, the solvent should not be considered as part of the substance.

Chrysanthemum cinerariaefolium extract from HCS, as pure active substance, has a minimum purity of 100% w/w. It is placed in the market as a solution, technical concentrate (c.a. 50% pyrethrins).

Table A.2 Structural formula

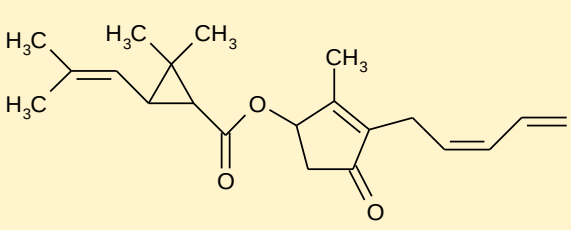
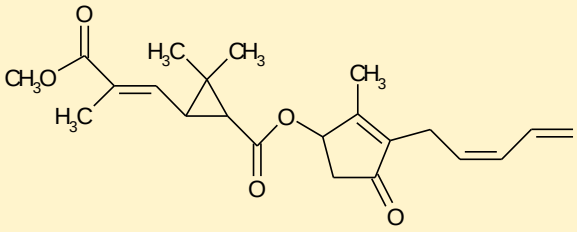
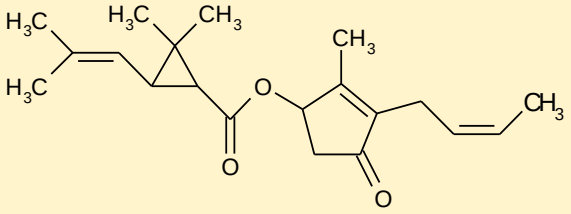
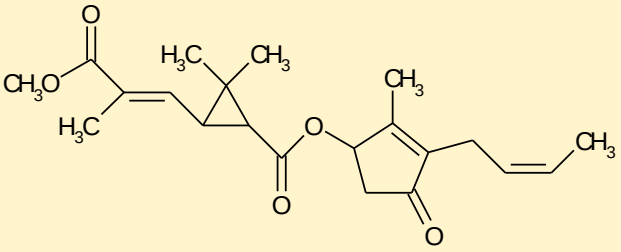
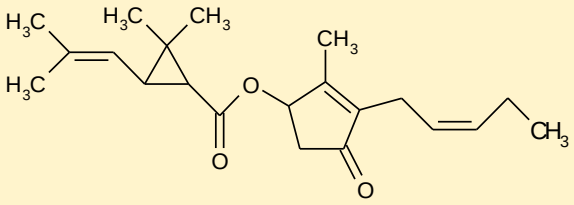
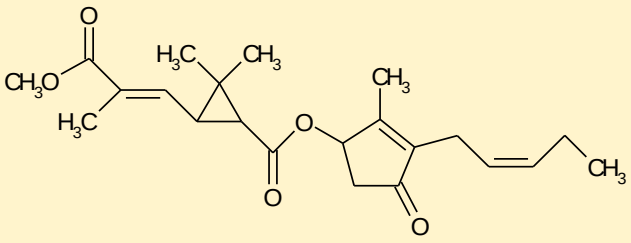
Structural formula
 pyrethrin 1
 pyrethrin 2
 cinerin 1
 cinerin 2
 jasmolin 1
 jasmolin 2

Table A.3 Origin of the natural active substance or precursor(s) of the active substance

Origin of the natural active substance or precursor(s) of the active substance
Chrysanthemum cinerariaefolium extract from HCS, pure, is an extract of the flower heads of *Chrysanthemum cinerariaefolium*. It contains Pyrethrins, which may be divided into the two groups: Pyrethrins I (consisting of pyrethrin 1, cinerin 1, and jasmolin 1)

and Pyrethrins II (consisting of pyrethrin 2, cinerin 2, and jasmolin 2).

A.1.2. Composition of the substance (reference specifications)

Table A.4 Constituents

Constituent(s)					
Constituent (chemical name)	Typical concentration (%(w/w))	Concentration range (%(w/w))	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	Remarks / Discussion
Chrysanthemum cinerariaefolium, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents (*Chrysanthemum cinerariaefolium* extract from HCS)	100% w/w	-	-	Acute Tox., Category 4; H302: Harmful if swallowed Acute Tox., Category 4; H332: Harmful if inhaled Skin sensitisation, Category 1B; H317: May cause an allergic skin reaction Short-term (acute) aquatic hazard, Category 1; H400: Very toxic to aquatic life Long-term (chronic) aquatic hazard, Category 1; H410: Very toxic to aquatic life with long lasting effects	Refer to the Appendix VI: Confidential Information for further details.

Table A.5 Impurities
This information can be found in the Appendix VI: Confidential Information.

Table A.6 Additives
The information can be found in the Appendix VI: Confidential Information.

Table A.7a Concentration of constituents in batches used for (eco)toxicity studies and proposed specification
All details are included in the relevant tables within Section A for this report.

Table A.8b Concentration of constituents in batches used for (eco)toxicity studies and proposed specification

All details are included in the relevant tables within Section A for this report.

A.1.3. Physical and chemical properties of the active substance

Table A.9 Physical and chemical properties of the active substance

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Aggregate state at 20°C and 101.3 kPA	-	-	-	-
Physical state (appearance) at 20°C and 101.3 kPA	Viscous liquid	-	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins)	A.3.3.1/01 Anonymous, 2001 (KPIC)
	Liquid	US EPA 63-3	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins)	Sundquist D.L., 1995 (MGK, BRA and SCJ)
	Clear Liquid	EPA/OCSPP 830.6303	Pure active substance (56.8% pyrethrins)	Comb, T., 2021 (MGK)
Colour at 20°C and 101.3 kPA	Amber	-	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins)	A.3.3.2/01 Anonymous, 2001 (KPIC)
	Clear, dark orange	US EPA 63-2	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins)	Sundquist D.L., 1995 (MGK, BRA and SCJ)

	Dark amber (10YR 7/8 on Munsell colour system)	EPA/OCSPP 830.6302	Pure active substance (56.8% pyrethrins)	Comb, T., 2021 (MGK)
Odour at 20°C and 101.3 kPA	Typical vegetable odour	-	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins)	A.3.3.3/01 Anonymous, 2001 (KPIC)
	Aromatic solvent-like odour	US EPA 63-4	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins)	Sundquist D.L., 1995 (MGK, BRA and SCJ)
	No discernible odour	EPA/OCSPP 830.6304	Pure active substance (56.8% pyrethrins)	Comb, T., 2021 (MGK)
Melting / freezing point	Pyrethrin 1: 142.3°C Pyrethrin 2: 132.1°C Cinerin 1: 135.2 ºC Cinerin 2: 124.2 ºC Jasmolin 1: 143.09 ºC Jasmolin 2: 133.25 ºC	EPIWIN QSAR	Determined by structure analysis. As the active substance is a plant extract, an experimental determination for the purified active substance is not possible.	A.3.1.1/01 Oellrich W., 2003 Note: study not used. (KPIC) Sundquist D.L., 1995 Note: study not used. (MGK, BRA and SCJ)

Boiling point	Pyrethrin 1: 400.8 ºC Pyrethrin 2: 421.8 ºC Jasmolin 1: 401.66 ºC Jasmolin 2: 422.67 ºC Cinerin 1: 390.06 ºC Cinerin 2: 411.07 ºC	EPIWIN QSAR	Determined by structure analysis. As the active substance is a plant extract, an experimental determination for the purified active substance is not possible.	A.3.1.2/01 Oellrich W., 2003 Note: study not used. (KPIC) Meinen V.J., 1988 Note: study not used. (MGK, BRA and SCJ)
	136-198ºC >210ºC	- -	Values derived from MSDS (KPIC) Values derives from MSDS (BRA, MGK and SCJ). In addition, decomposition of the active substance as manufactured (ca 50% pyrethrins) starts at temperatures above 270ºC.	- -
Acidity	Not applicable	-	Study not required. Not relevant, the active substance is not water-solvent based.	-
Relative density	0.97-0.98 g/cm^3		Plant extract, experimental determination for purified active substance not possible, values derived from specification data sheet. (Active substance as manufactured, technical concentrate, TK) (ca 50% pyrethrins)	A.3.1.3/01 Anonymous, 2000 (KPIC)

	Rel. Density: $\rho^{19.6}{}_{4.0}$: 0.962	EEC A.3, OECD 109	(Active substance as manufactured, technical concentrate, TK) (ca 50% pyrethrins)	A.3.1.3/02 Walter, 2008a (KPIC)
	0.952 g/mL at 20ºC $D^{20}{}_4$ = 1.00	US EPA 63-7 EC Method A.3, OECD Method 109 and EPA/OCSPP 830.7300	(Active substance as manufactured, technical concentrate, TK) (ca 50% pyrethrins) Pure active substance (56.8% pyrethrins)	Sundquist D.L., 1995 (MGK, BRA and SCJ) Comb, T., 2021 (MGK)
Absorption spectra data (UV/VIS, IR, NMR) and a mass spectrum	UV spectra confirmed the chemical structure. UV-Vis absorption Acid medium: max 417 nm, Epsilon 126 max 226 nm, Epsilon 5432 max 290 nm; Epsilon 295 Basic Medium: max 442 nm, Epsilon 121 max 229 nm, Epsilon 4790 max 290 nm; Epsilon 226	OECD 101	Pyrethrum extract (purity not stated)	Schmid J., 1990 (KPIC)

	IR spectra confirmed the chemical structure. C-H stretching vibration: 3000-2800 cm^{-1} C=O stretching vibration: 1707 cm^{-1} CH_2sym CH_3 sy: 1454 cm^{-1} C $(CH_3)_2$: 1372, 1358 cm^{-1} C-O: 1147 cm^{-1}	Not stated	Pyrethrum Extract 25%, batch no. 983/pale	Werle H., 1994 (KPIC)
	NMR spectra confirmed the chemical structures.	Not stated	Pyrethrin 1, cinerin1, jasmolin 1, pyrethrin 2, cinerin 2, jasmolin 2	Casida J.E., 1973 (KPIC)
	GC-MS spectra confirmed the chemical structures. cinerin 1: m/z 81, 93, 123, 150, 316 jasmolin 1: m/z 81, 93, 123, 164, 330 pyrethrin 1: m/z 81, 91, 123, 133, 162, 168, 328 cinerin 2: m/z 93, 107, 121, 149, 167 jasmolin 2: m/z 107, 121, 135, 163, 167, 212 pyrethrin 2: m/z 133, 161, 167, 372	Not stated	Pyrethrin 1, cinerin1, jasmolin 1, pyrethrin 2, cinerin 2, jasmolin 2	Bruske L.J., 1987 (KPIC)
	UV-Vis absoroption The test substance is not a pure material; therefore, it is impossible to calculate the molar extinction coefficients for the absorbance maxima. The contribution of each component in	US EPA OPPTS 830.7050	Premium PYROCIDE 175 (20%), bacth No. 4AE10421 (isopropanol solution)	Sinning D.J., 2002 (MGK, BRA and SCJ)

	the test substance to each absorbance maximum, which is located between 225 and 230 nm - could not be separated.			
	UV-Vis absorption Ethanol solvent: Pyrethrin 1: max 225 nm, Epsilon 36420 Cinerin 1: max 226 nm; Epsilon 17720 Pyrethrin 2: max 229 nm; Epsilon 45850 Cinerin 2: 227-9 nm; Epsilon 28946 n-hexane solvent: Pyrethrin 1: max 222 nm; Cinerin 1: max 220 nm Pyrethrin 2: max 227-9 nm Cinerin 2: max 226-8 nm	Not stated	Natural Pyrethrins purified using a Celite column; Purity: Not specified; Batch number: Not specified	Chang S.C., 1961 (MGK, BRA and SCJ)
	UV-Vis absorption Pyrethrin 1: max 222.5 nm; Epsilon 38800 Cinerin 1: max 221 nm; Epsilon 21100 Pyrethrin 2: max 228 nm; Epsilon 47500 Cinerin 2: max 229 nm; Epsilon 27900	Not stated	Main biologically active components pyrethrins separated from commercial Pyrethrum extracts; Purity: Not specified; Batch number: Not specified (n-hexane soultions)	Sawicki R.M. & Thain E.M., 1961 (MGK, BRA and SCJ)
	IR Absorbance	Not stated	Natural pyrethrum extract	Moorman R. &

	Pyrethrin 1: 2929 cm^{-1}: C-H asym; 1735 cm^{-1}: C=O; 1655 cm^{-1}: C=C; 1148 cm^{-1}: C-O stretch; 910 cm^{-1}: C-H out of the plane Cinerin 1: 2929 cm^{-1}: C-H asym; 2880 cm^{-1}: C-H sym; 1735 cm^{-1}: C=O; 1657 cm^{-1}: C=C; 1148 cm^{-1}: C-O stretch Jasmolin 1: 2932 cm^{-1}: C-H asym; 2882 cm^{-1}: C-H sym; 1735 cm^{-1}: C=O; ~1650 cm^{-1}: C=C; 1148 cm^{-1}: C-O stretch Pyrethrin 2: 2980 cm^{-1}: C-H asym; 1735 cm^{-1}: C=O; 1655 cm^{-1}: C=C; 1327, 1220, 1150 and 1055: C-O stretch; 910 cm^{-1}: C-H out of the plane Cinerin 2: 2936 cm^{-1}: C-H asym; 2886 cm^{-1}: C-H sym; 1735 cm^{-1}: C=O; 1657 cm^{-1}: C=C; 1148 cm^{-1}: C-O stretch Jasmolin 2: 2976 cm^{-1}: C-H asym; 2889 cm^{-1}: C-H sym; 1735 cm^{-1}: C=O; ~1650 cm^{-1}: C=C; 1148 cm^{-1}: C-O stretch		(19.26%); Batch number: Not specified (isopropanol solutions)	Nguyen K.T., 1997 (MGK, BRA and SCJ)
	IR Absorbance Pyrethrins I and II: 905 cm^{-1}: =CH2 terminal, CH out of the plane; Absorption at 800-1800 cm^{-1}.	Not stated	Pyrethrum extracts 25% (Belgian Congo and Kenya); Batch number: Not specified Method used: Not stated	Sawicki R. M. & Thain E. M., 1961 (MGK, BRA and SCJ)
	IR Absorbance Pyrethrin 1: 1703 cm^{-1}: C=O	Not stated	Purified natural pyrethrins and reconstituted esters;	Elliott M., 1961 (MGK, BRA and SCJ)

	1282, 1227, 1198 and 1149 cm^{-1}: C-O stretch 909 cm^{-1}: CH out of the plane 855 cm^{-1}: CH out of the plane Cinerin 1: 1703 cm^{-1}: C=O 1282, 1227, 1198 and 1149 cm^{-1}: C-O stretch 855 cm^{-1}: CH out of the plane Pyrethrin 2: 1703 cm^{-1}: C=O 1605 cm^{-1}: C=C 1266, 1220, 1170,1149 cm^{-1}: C-O strech 909 cm^{-1}: CH out of the plane 756 cm^{-1}: CH out of the plane Cinerin 2: 1703 cm^{-1}: C=O 1605 cm^{-1}: C=C 1266, 1220, 1170, 1149 cm^{-1}: C-O stretch 756 cm^{-1}: CH out of the plane		Purity: Not specified; Batch number: Not specified	
	The spectra of the NMR analysis of Pyrethrin 1 and 2 indicate that the structures of purified Pyrethrins are consistent with the proposed structures.	Not stated	Natural pyrethrins separated from World Standard Pyrethrum Extract (1974); Purity: Not specified; Batch number: Not specified	Dickinson C.M., 1982 (MGK, BRA and SCJ)
	MS Pyrethrin 1: m/z 168, 162, 133, 123, 105, 91 Cinerin 1: m/z 168, 150, 123, 107, 105, 93, 91	Not stated	19.26% pure Natural pyrethrum extract; Batch number: Not specified	Moorman R. & Nguyen K.T., 1997 (MGK, BRA and SCJ)

	Jasmolin 1: m/z 164, 162, 133, 123, 107, 105, 93, 91 Pyrethrin 2: m/z 167, 160, 145, 133, 119, 107, 105, 91 Cinerin 2: m/z 167, 149, 121, 107, 105, 93, 91 Jasmolin 2: m/z 167, 162, 133, 121, 107, 105, 93, 91			
	MS Pyrethrin 1: Ester moiety m/z 329 [MH+] m/z 357 [M+C_2H_5]+ Acid moiety m/z 169 [RC$(OH)_2$]+ m/z 151 [RCO]+ m/z 123 [R]+ Alcohol moiety m/z 189 [R+ C_2H_4]+ m/z 161 [R]+ Cinerin 1: Ester moiety m/z 317 [MH]+ m/z 345 [M+C_2H_5]+ Acid moiety m/z 169 [RC$(OH)_2$]+	Not stated	Pyrethrins from purified 72% Pyrethum extract; Batch number: Not specified	Class T.J. *et al.*, 1989 (MGK, BRA and SCJ)

	m/z 151 [RCO]+ m/z 123 [R]+ Alcohol moiety m/z 177 [R+ C_2H_4]+ m/z 149 [R]+ Jasmolin 1: Ester moiety m/z 331 [MH]+ m/z 359 [M+C_2H_5]+ Acid moiety m/z 169 [RC(OH)$_2$]+ m/z 151 [RCO]+ m/z 123 [R]+ Alcohol m/z 191 [R+ C_2H_4]+ m/z 163 [R]+ Pyrethrin 2: Ester moiety m/z 373 ester [MH]+ m/z 401 [M+C_2H_5]+ m/z 341 [MH-CH_3OH]+ Acid moiety m/z 213 [RC(OH)$_2$]+ m/z 195 acid [RCO]+ m/z 167 acid [R]+ Alcohol moiety			

	m/z 189 [R+ C_2H_4]+ m/z 161 [R]+ Cinerin 2: Ester moiety m/z 361 [MH]+ m/z 389 [M+C_2H_5]+ m/z 329 [MH-CH3OH]+ Acid moiety m/z 213 [RC$(OH)_2$]+ m/z 195 [RCO]+ m/z 167 [R]+ Alcohol m/z 177 [R+ C_2H_4]+ m/z 149 [R]+ Jasmolin 2: Ester moiety m/z 375 [MH]+ m/z 403 [M+C_2H_5]+ m/z 343 [MH-CH_3OH]+ Acid moiety m/z 213 [RC$(OH)_2$]+ m/z 195 [RCO]+ m/z 167 [R]+ Alcohol moiety m/z 165 [R+ C_2H_4]+ m/z 163 [R]+			

			No determination will be performed using purified active substance, as the data provided relating to the individual pyrethrins is not impacted by the presence or absence of other components.	(MGK)
Vapour pressure	Pyrethrin 1: 6.9E-05 Pa (25°C) Pyrethrin 2: 2.7E-05 Pa (25°C) Jasmolin 1: 6.4E-05 Pa (25°C) Cinerin 1: 1.5E-04 Pa (25°C) Jasmolin 2: 2.5E-05 Pa (25°C) Cinerin 2: 6.1E-05 Pa (25°C)	Episuite "Mpbpvp", MPBPWIN 1.42, US EPA, 2000	Determined by structure analysis. As the active substance is a plant extract, an experimental determination for purified active substance is not possible	A.3.2/01 Oellrich W., 2003 (KPIC)
	Due to the differences between the pure active substances and the specification of the Pale Extract, the calculated and experimental values for the vapour pressure of Pyrethrins are different	EpiSuite MPBPWIN 1.42, US EPA, 2000	Statement on differences between calculated and experimental values for the vapour pressure of Pyrethrins.	A.3.2/03 Tiemann, 2004 (KPIC)
	293 Pa (20°C) 453 Pa (50 ºC)	ASTM part 23, D323-82; equivalent to DIN 51754	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins)	A.3.2/02 Anonymous (date not given) (KPIC)
	Pyrethrins: 3.13E-05 Pa Pyrethrin 1: 1.88E-04 Pa Pyrethrin 2: 1.21E-05 Pa Cinerin 1: 6.93E-05 Pa	EpiSuite MPBPWIN, US EPA, 2008	-	O'Carroll N., 2008 (MGK, BRA and SCJ)

	Cinerin 2: 2.77E-05 Pa Jasmolin 1: 2.92E-05 Pa Jasmolin 2: 1.12E-05 Pa Temperature: 20ºC			
Henry's law constant	pyrethrin 1: 7.83E-02 Pa·m^3/mol at 25°C pyrethrin 2: 7.19E-05 Pa·m^3/mol at 25°C jasmolin 1: 1.29E-01 Pa·m^3/mol at 25ºC jasmolin 2: 1.23E-04 Pa·m^3/mol at 25ºC cinerin 1: 9.72E-02 Pa·m^3/mol at 25ºC cinerin 2: 9.29E-05 Pa·m^3/mol at 25ºC	Determination by calculation method.	In the absence of experimental data for each main compound, these HLC values calculated via bond method should be deemed as more accurate.	A.3.2.1/01 Oellrich W., 2001 (KPIC)
	Pyrethrin 1: 0.268 Pa m^3/mol Pyrethrin 2: 0.0626 Pa m^3/mol Temperature: 20ºC	Calculated	-	O'Carroll N., 2008 (MGK, BRA and SCJ)
			No determination will be performed using purified active substance, as no new vapour pressure or water solubility data are being generated.	(MGK)
Surface tension	52.8 mN/m at 20°C	EU Method A.5 (Surface Tension)	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins)	A.3.13/01 Walter D., 2005 (KPIC)

			The water solubility of the experimental and estimated values of the known constituents (>90%) indicate that a surface tension test is not required.	(MGK, BRA and SCJ)
	56.0 mN/m at 20 ºC	EC Method A.5 and OECD Method 115 (harmonised ring method)	Pure active substance (56.8% pyrethrins) 90% saturated test item solution used	Comb, T., 2021 (MGK)
Water solubility at 20°C	pyrethrin 1: 0.2 mg/L pyrethrin 2: 9 mg/L cinerin 1: 0.6 mg/L cinerin 2: 0.301 mg/L jasmolin 1: 0.027 mg/L jasmolin 2: 0.094 mg/L (all values measured at 25ºC)	Not stated	Pyrethrum Extract (Lot No. not stated; purity not stated)	A.3.5/01 Pfersich, 2001 (KPIC)
	pyrethrin 1: pH 5: 0.57 mg/L pH 7: 0.96 mg/L pH 10: 0.44 mg/L pyrethrin 2: pH 5: 8.2 mg/L pH 7: 10.7 mg/L pH 10: 7.9 mg/L	CIPAC MT 157.1	Pyrethrum Extract (Lot No. not stated; purity not stated)	A.3.5/02 Schmid, 1990 (KPIC)

	(all values measured at room temperature)			
	Determined at 20ºC Pyrethrin 1: 0.23 mg/L Pyrethrin 2: 0.072 mg/L Estimated for 25ºC Jasmolin 1: 0.027 mg/L Cinerin 1: 0.085 mg/L Jasmolin 2: 0.094 mg/L Cinerin 2: 0.301 mg/L	US EPA D-63-8, OPPTS 830.7840	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins) No determination will be performed using test material without solvent, as the active substance is a plant extract. An experimental determination for the purified active substance is therefore not possible. Data for the individual pyrethrins has already been provided.	Sinning D.J., 2005 (MGK, BRA and SCJ) (MGK)
Partition coefficient (n-octanol/water) and its pH dependency	Log Pow (n-octanol/water): Pyrethrin 1: 5.59 Cinerin 1: 5.54 Jasmolin 1: 6.04	OECD 117 (HPLC method)	Reference standard (≥ 97%) Lots: XX8-82-P1	Mori V., 2015 (KPIC, MGK, BRA and SCJ)

	Pyrethrin 2: 4.32 Cinerin 2: 4.26 Jasmolin 2: 4.74		XX8-82-C1 XX8-82-J1 XX8-82-P2 XX8-82-C2 XX8-82-J2	
	Pyrethrin 1: Log Kow = 5.34 (20˚C) Pyrethrin 2: Log Kow = 3.79 (20˚C) Jasmolin 1: Log Kow = 6.42 (25˚C) Jasmolin 2: Log Kow = 5.47 (25˚C) Cinerin 1: Log Kow = 5.93 (25˚C) Cinerin 2: Log Kow = 4.98 (25˚C)	EU Method A.8 (Partition Coefficient)	World Standard Pyrethrum Extract	A.3.9/01 Ochieng, 1990 (KPIC)
	Pyrethrin 1: 5.9 Pyrethrin 2: 4.3 Cinerin 1: 5.6 Estimated at 25 ºC Jasmolin 1: 6.42 Jasmolin 2: 5.47 Cinerin 2: 4.98	Not stated	Pyrethrum Extract (purity and lot No. not stated) Effect of pH is not relevant as the test substance is neither acidic nor basic.	Briggs, G.G., Elliott M., Janes N.F., 1982 (MGK, BRA and SCJ)
			No determination will be performed using purified active substance, as the active substance is a plant extract. An experimental determination for the purified active substance is therefore not possible. Data for the individual pyrethrins	(MGK)

			has already been provided.	
Thermal stability and identity of breakdown products			The active substance is a plant extract including a lot of single compounds. An identification of breakdown products during thermal decomposition is not possible. No major changes of the content of a.i. in the formulations after storage for 14 days at 54°C have been observed. The corresponding reports are submitted in Section B.	(KPIC)
	Thermally stable at room temperature below 150ºC. Oxidative decomposition underwent at temperatures above 270ºC.	OECD Guideline 113 (Differential Scanning Calorimetry)	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins) The results from tests in air showed the initiation of predominant exothermic effects at temperatures above 270°C indicating significant decomposition of the test substance. The decomposition was subsequently confirmed by the presence of a black residue on completion of the tests.	Comb A.L., 2008 (MGK, BRA and SCJ)

			In comparison, the results from the tests in nitrogen showed only a slight increase in the baseline which was consistent with the heating of the apparatus characteristically observed for all samples using the specified apparatus. It should be noted that the scale of the thermal assessment of the nitrogen samples is much smaller than the traces presented for the air samples. The conclusion from these observations was that the sample predominantly underwent oxidative decomposition at temperatures above 270°C	
Reactivity towards container material	Diluted pyrethrum extract was demonstrated to react with metals iron, copper, zinc and lead. Tin did not react with the pyrethrum extract.	Not stated	Pyrethrum Extract (25%) refined Pale product in Shellsol T	Chiu F.T. & Wu N.C., 1974 (MGK, BRA and SCJ)
	Stable for 1 year at 100°F (37.8°C) in F-Style Tin and FL-HDPE. Stable for 1 year at room temperature in amber glass.	US EPA 63-17	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins)	Sundquist D. L., 1996 (MGK, BRA and SCJ)
Dissociation constant	Not applicable	-	Pyrethrins are not able to dissociate in water.	-

Granulometry	Not applicable	-	The active substance is a liquid.	-
Viscosity	At 20°C: 135 mPa s At 40°C: 36 mPa s	OECD Test Guideline 114 (Viscosity of Liquids) CIPAC MT 192	The test item can be considered as Newtonian liquid because the shear rate is nearly constant. (Active substance as manufactured, technical concentrate, TK) (ca 50% pyrethrins)	A.3.14/02 Walter, 2008b (KPIC)
	85.5 mPa s at 23 ºC	US EPA 63-18	(Active substance as manufactured, technical concentrate, TK) (ca 50% pyrethrins)	Sundquist D.L., 1995 (MGK, BRA and SCJ)
	380 mPa.s at 20°C (shear rates approx. 25 – 75 rpm) 75 mPa.s at 40°C (shear rates approx. 50 – 150 rpm)	OECD Method 114, EPA/OCSPP 830.7100 and CIPAC MT 192 (rotational viscometer)	Pure active substance (56.8% pyrethrins) As there was no significant change in the viscosity readings with shear rate, it can be concluded that the test item is a Newtonian liquid.	Comb, T., 2021 (MGK)
Solubility in organic solvents, including	n-heptane: > 250 g/L (20°C) p-xylene: > 250 g/L (20°C)	CIPAC MT 181 The solubility of a	Active substance as manufactured (technical	A.3.7/01 Walter D., 2000 (KPIC)

effect of temperature on solubility	1,2-dichloroethane: > 250 g/L (20°C) methanol: > 250 g/L (20°C) acetone: >250 g/L (20°C) ethylacetate: >250 g/L (20°C)	test substance in organic solvent was determined by adding successive measured volumens of solvents to a known mass of the test substance until complete dissolution was observed	concentrate, TK) (ca 50% pyrethrins)	
	Hexane: >65 g/L Methanol: 45 g/L Xylenes: >56 g/L Acetone: > 65 g/L Dichloromethane: >65 g/L Temperature: 20^0C	Not stated. Approximately 10 g of test substance was mixed with 100 mL of solvent. The solutions were allowed to stand at 20ºC overnight. The resulting solution was filtered through 0.45 micron filter. A 10 mL alicuot was prepared for analysis by heating to remove the solvent by	Active substance as manufactured (technical concentrate, TK) (ca 50% pyrethrins)	Faber H., 2004 (MGK, BRA and SCJ)

		evaporation. The residual was re-dissolved in hexane. Pyrethrins were determined by HPLC.		
Stability in organic solvents used in biocidal products and identity of relevant degradation products	Stable in isopropanol, petroleum distillate and propylene glycol at 5% for 2 weeks at 54ºC	CPT-2164	5.0% pure Pyrethrum Concentrate; Batch number: Not specified	Bergman J.T., 2007 (MGK, BRA and SCJ)

Conclusion on the physical, chemical and technical properties of the active substance

Physico-chemical properties of *Chrysanthemum cinerariaefolium* extract from HCS were initially determined on the whole extract (i.e. in the active substance as manufactured (technical concentrate), with the solvent present). The solvent reduces the viscosity of the extract to make it easier to pour and mix during formulation, and also helps to keep the stabilizer in solution (stabilizer is added to avoid oxidation of the pyrethrins). However, the solvent should not be part of the active substance, since the stability study shows that *Chrysanthemum cinerariaefolium* extract from HCS remains stable without the solvent, showing only a slight decrease in stability, which is not enough to support the inclusion of the solvent in the composition of the active substance.

Therefore, some physico-chemical properties (appearance – physical state, colour and odour -, relative density, surface tension and viscosity) have been tested again using the pure extract, without the solvent. Other physico-chemical endpoints are already sufficiently addressed or are technically not possible to determine using the pure active substance.

Chrysanthemum cinerariaefolium extract from HCS (pure active substance) is a dark amber liquid with no discernible odour. Its relative density D_4^{20} is 1 and its surface tension is 56.0 mN/m at 20 ºC. The viscosity is 380 mPa.s at 20°C and 75 mPa.s at 40°C and it can be considered a Newtonian liquid.

Physico-chemical properties are, in general, determined on the whole extract, and not on individual constituents.

ES	*Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

A.1.4. Physical hazards and respective characteristics

Table A.10 Physical hazards and respective characteristics

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
Explosives	-	-	The molecular structure of Pyrethrins indicates that the substance has little or no explosive properties. However, this waiving is not acceptable. Structural considerations are insufficient for UVCB substances to conclude on this physical hazard.	(BRA, MGK and SCJ)
	UN Recommendations on the Transport of Dangerous Goods, 7th Ed., 2019, Appendix 6	DSC Screening determination for heat of decomposition	Pure active substance (56.8% pyrethrins) DSC Exotherms: Exotherm onset temp (ºC) 1st: 149.03 2nd: 253.51 Heat of decomposition (J/g) 1st: 34.908 2nd: 41.191	Siusiene, E., 2022 (MGK)

ES *Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			Chrysanthemum cinerariaefolium extract from HCS is not a candidate for classification as a UN Class 1 explosive substance as the total heat of decomposition was <500 J/g.	
	EU Method A.14 (Explosive properties)	DSC Screening test was performed.	The test substance shows an exothermal effect in the range 250-440°C with a decomposition energy of 354 J/g. The decomposition energy is below 500 J/g, therefore the main test is unnecessary. No risk of explodability. (Active substance as manufactured, technical concentrate, TK) (ca 50% pyrethrins)	Hoffmann H., 2000 (KPIC)
Flammable gases	-	-	Not applicable	-
Flammable aerosols	-	-	Not applicable	-
Oxidising gases	-	-	Not applicable	-
Gases under pressure	-	-	Not applicable	-
Flammable liquids	ASTM D 56 - Non-equilibrium ISO 1523 - Equilibrium	-	The flash point of the active substance is 72°C, measured with the non-equilibrium method (ASTM D56) and 71°C with the equilibrium method (ISO 1523).	Bergman J.T., 2008 (BRA, MGK and SCJ)

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			(Active substance as manufactured, technical concentrate, TK) (ca 50% pyrethrins)	
	EU Method A.9 (Flash Point)	Pensky-Martens apparatus	Flash point 69.4°C. (Active substance as manufactured, technical concentrate, TK) (ca 50% pyrethrins)	Walter D., 2000 (KPIC)
	EU Method A.9 (Flash Point)	Pensky-Martens apparatus (closed cup)	Pure active substance (56.8% pyrethrins) Flash point = 146.5 ºC *Chrysanthemum cinerariaefolium* extract from HCS is not flammable.	Siusiene, E., 2022 (MGK)
Flammable solids	-	-	Not applicable	-
Self-reactive substances and mixtures	-	-	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. This waiving is not acceptable. Structural	-

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			considerations are insufficient for UVCB substances to conclude on this physical hazard.	
	UN Recommendations on the Transport of Dangerous Goods, 7th Ed., 2019, Appendix 6	DSC Screening determination for heat of decomposition	Pure active substance (56.8% pyrethrins) DSC Exotherms: Exotherm onset temp (ºC) 1st: 149.03 2nd: 253.51 Heat of decomposition (J/g) 1st: 34.908 2nd: 41.191 *Chrysanthemum cinerariaefolium* extract from HCS is not a candidate for classification as a UN Class 4, Division 4.1 self-reactive substance as the total heat of decomposition was <300 J/g.	Siusiene, E., 2022 (MGK)
Pyrophoric liquids	-	-	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	-

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			(days) and hence the classification procedure does not need to be applied.	
Pyrophoric solids	-	-	Not applicable	-
Self-heating substances and mixtures	-	-	Not applicable as the substance is a liquid.	-
Substances and mixtures which in contact with water emit flammable gases	-	-	The study does not need to be conducted because the organic substance does not contain metals or metalloids and hence the classification procedure does not need to be applied.	-
	UN Test N.5	Spontaneous ignition with water/evolution of flammable gas > 1L/kg/hr	Pure active substance (56.8% pyrethrins) No gas generation or spontaneous ignitions observed. Classified as not UN Division 4.3	Siusiene, E., 2022 (MGK)
Oxidising liquids	-	-	The molecular structure of Pyrethrins indicates that there is little or no potential for this material as an oxidising or reducing agent. However, this waiving is not acceptable. Structural considerations are insufficient for UVCB substances to conclude on	(BRA, MGK and SCJ)

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			this physical hazard.	
	QSAR	Determined by structure analysis of the main components of the active substance and using the model MPBPWIN.	Following the structures of Pyrethrins, any oxidizing properties can be excluded	Oellrich W., 2002 (KPIC)
	UN Test O.2	Mean pressure rise	Pure active substance (56.8% pyrethrins) The 1:1 mixture of the test item and cellulose was observed to have a mean pressure rise time greater than that of a 1:1 mixture of 65 % nitric acid and cellulose. The test item is therefore exempt from classification as an oxidising liquid of UN Class 5, Division 5.1	Siusiene, E., 2022 (MGK)
Oxidising solids	-	-	Not applicable	
Organic peroxides	-	-	This 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.	-
Corrosive to metals	-	-	The study does need to be conducted since based on the chemical evaluation none of	-

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			the components contain chemical groups, which could initiate an irreversible electrochemical reaction with metals leading to significant damage or destruction. However, this waivng is not acceptable, as it is not based on CLP.	
	UN Test C.1	Corrosion to Metals (mass loss & pitting using steel and aluminium coupons)	Pure active substance (56.8% pyrethrins) The percentage mass losses on steel and aluminium were found to be <13.5 % over 7 days and no pitting was observed. *Chrysanthemum cinerariaefolium* extract from HCS is therefore exempt from classification as a corrosive substance of UN Class 8, Packing group III (according to the UN Transport of Dangerous Goods Recommendations)	Siusiene, E., 2022 (MGK)
Auto-ignition temperature (liquids and gases)	EU Method A.15 (Auto-Ignition Temperature (Liquids and	-	The self-ignition temperature of the active substance is 270°C. (Active substance as	Hoffmann H., 2000 (KPIC)

ES *Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
	Gases))		manufactured, technical concentrate, TK) (ca 50% pyrethrins)	
			The product is not expected to be flammable according to the flash point and the explosives and oxidising properties. This waiving is not acceptable, as it is not based on CLP.	(MGK, BRA and SCJ)
	EU Method A.15 (Auto-Ignition Temperature (Liquids and Gases))		Pure active substance (56.8% pyrethrins) The autoignition temperature of *Chrysanthemum cinerariaefolium* extract from HCS has been determined to be 284°C.	Siusiene, E., 2022 (MGK)
Relative self-ignition temperature for solids	-	-	Not applicable	-
Dust explosion hazard	-	-	Not applicable	-

Conclusion on the physical hazards and respective characteristics of the active substance

Physical hazards of *Chrysanthemum cinerariaefolium* extract from HCS were initially determined on the whole extract (i.e. in the active substance as manufactured (technical concentrate), with the solvent present). However, the solvent is not part of the active substance composition. Furthermore, some physical hazards were waived based on the chemical structure of the active substance, but structural considerations in UVCB substances are not acceptable.

Therefore, physical hazard tests have been repeated on the purified active substance, without the solvent.

Chrysanthemum cinerariaefolium extract from HCS (pure active substance) is not considered explosive nor self-reactive. It is not flammable nor oxidising. It does not emit flammable gases in contact with water. It is not corrosive to metals.

In conclusion, no physical hazard has been identified for *Chrysanthemum cinerariaefolium* extract from HCS (pure active substance).

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The following hazard classes are considered by the DS as not applicable:

- Flammable gases
- Flammable aerosols
- Oxidising gases
- Gases under pressure
- Flammable solids
- Pyrophoric solids
- Self-heating substances and mixtures
- Oxidising solids
- Organic peroxides

Explosives

The DS proposed no classification because the total heat of decomposition of the substance is <500 J/g and its onset temperature is <500°C. [CLP §2.1.4.3 c]

Flammable liquids

The DS proposed no classification because the flash point of the substance is above the classification threshold of 60°C. [CLP §2.6.1]

Self-reactive substances and mixtures

The DS proposed no classification because the total heat of decomposition of the

substance is <300 J/g. [CLP §2.8.2.1]

Pyrophoric liquids

The DS proposed no classification based on the experience in manufacturing and handling that shows no spontaneous ignition in contact with air at normal temperature for prolonged periods of time. [CLP §2.9.4.1]

Substances and mixtures which in contact with water emit flammable gases

The DS proposed no classification due to the absence of gas generation or spontaneous ignitions observe when using the UN Test N.5 method. [CLP §2.12.2.2.1]

Oxidising liquids

The DS proposed no classification because the results of an UN O.2 test concluded to the exclusion of the substance from Division 5.1. [CLP §2.13.2.1]

Corrosive to metals

The DS proposed no classification because the results of an UN C.1 test concluded to the exclusion of the substance from classification as a corrosive substance of UN Class 8. [CLP §2.16.2.1]

Comments received during consultation

During the consultation, one MS questioned the auto-ignition temperature of 284 °C determined for the pure active substance (Siusiene, 2022) and asked for clarification about the measured auto-ignition temperature that may not correspond to the auto-ignition temperature of the substance. The DS considered that as the conditions used to conduct the DSC screening and the AIT are very different and they may not be comparable. As a UVCB with a multitude of constituents, not only pyrethrins, the energetic activity showed in the DSC and the measured autoignition temperature may be influenced by different constituents present in the mixture.

Assessment and comparison with the classification criteria

RAC agrees with the DS assessment for all physical hazard classes. No physical hazard is identified and **none of the physical hazard classes opened for assessment warrant classification**.

A.1.5. Assessment of physical hazards according to the CLP criteria

A.1.5.1. *Assessment of physical hazards*

Chrysanthemum cinerariaefolium extract from HCS (pure active substance) does not meet any physical hazard class described in BPR.

A.2. Assessment of effects on Human Health

A.2.1. Toxicokinetics

A2.1.1 Short summary and overall relevance of the provided toxicokinetic information

Toxicokinetics and distribution

^{14}C-Pyrethrin was administered orally to male and female Sprague-Dawley rats. In a preliminary study time peak blood level and blood half-life were determined. In three definitive experiments, absorption, distribution, excretion and metabolism were examined (Selim, 1995). The definitive studies were conducted using three groups of 5 rats/sex/group as follows: a single low-dose (Group 1: 10 mg/kg bw), a single high-dose (Group 2: 50 mg/kg bw for females and 100 mg/kg for males) and a repeated low-dose (Group 3: non-radiolabelled Pyrethrin 1 at 10 mg/kg bw for 14 days prior to administration of a single oral low-dose of ^{14}C-Pyrethrin 1 on day 15). Two additional groups of 5 rats/sex/group were dosed exclusively for the purposes of isolation and identification of metabolites. In this ^{14}C-Pyrethrin 1 was administered orally at a single oral low-dose (Group 4: 10 mg/kg bw) and at a single oral high dose (Group 5: 50 mg/kg bw for females and 100 mg/kg for males).

All rats survived the studies and no signs of toxicity were observed. The preliminary study showed that less of the administered ^{14}C-Pyrethrin 1 was systemically absorbed by the male rats than by the female rats with peaks in blood after 5-6 and 6-8 hours, respectively. Male rats excrete the ^{14}C-Pyrethrin 1 derived radioactivity faster than females, since the elimination half-time in males and females was 5.3 and 6.7 hours, respectively.

In all groups of the definitive studies the majority of radioactivity was excreted in the urine and faeces during the first 72 hours following administration. The levels of radioactivity expired as $^{14}CO_2$ were very low. Regardless of dosage regimen, males excrete a majority of the administered radioactivity in the faeces via the enterohepatic circulation while females excrete approximately equal amounts in the urine and faeces. Repeated administration of Pyrethrin 1 increased the rate of elimination of the ^{14}C-Pyrethrin 1-derived radioactivity from the body in both males and females thus suggesting that repeated dose of ^{14}C-Pyrethrin 1 results in an induction of the liver microsomal enzyme system.

Analysis of tissues at 7 days showed that in all dosing regimens, concentrations of radioactivity more than twice that of whole blood were found in liver, ovaries, carcass and fat. The radioactive residues in the fat were consistently higher than the residues in all other tissues analysed. The elimination of substantial amounts of ^{14}C-Pyrethrin 1-derived radioactivity in the faeces over and extended length of time indicated that enterohepatic circulation played a role in the elimination of the compound and/or its metabolites from the body.

Metabolism

In the metabolism study, two additional groups of male and female rats were orally dosed with ^{14}C-Pyrethrin 1 at single low-dose and single high-dose for isolation and identification of metabolites in urine and faeces. This study demonstrated that ^{14}C-Pyrethrin 1 is metabolized to six compounds, having been identified two major metabolites (Metabolite C and E) along with 4 minor metabolites (A, B, D, and F). The results indicated that in the rat, Pyrethrin 1 is metabolized by two major metabolic pathways. One pathway is by oxidation of the double bond on the cyclopentene or the cyclopropane side of the molecule to form a diol, in addition to oxidation of the methyl groups on the side chain of the cyclopropane ring to form a carboxylic acid. The second pathway is by hydrolysis of the ester bond to form the corresponding acid and alcohol.

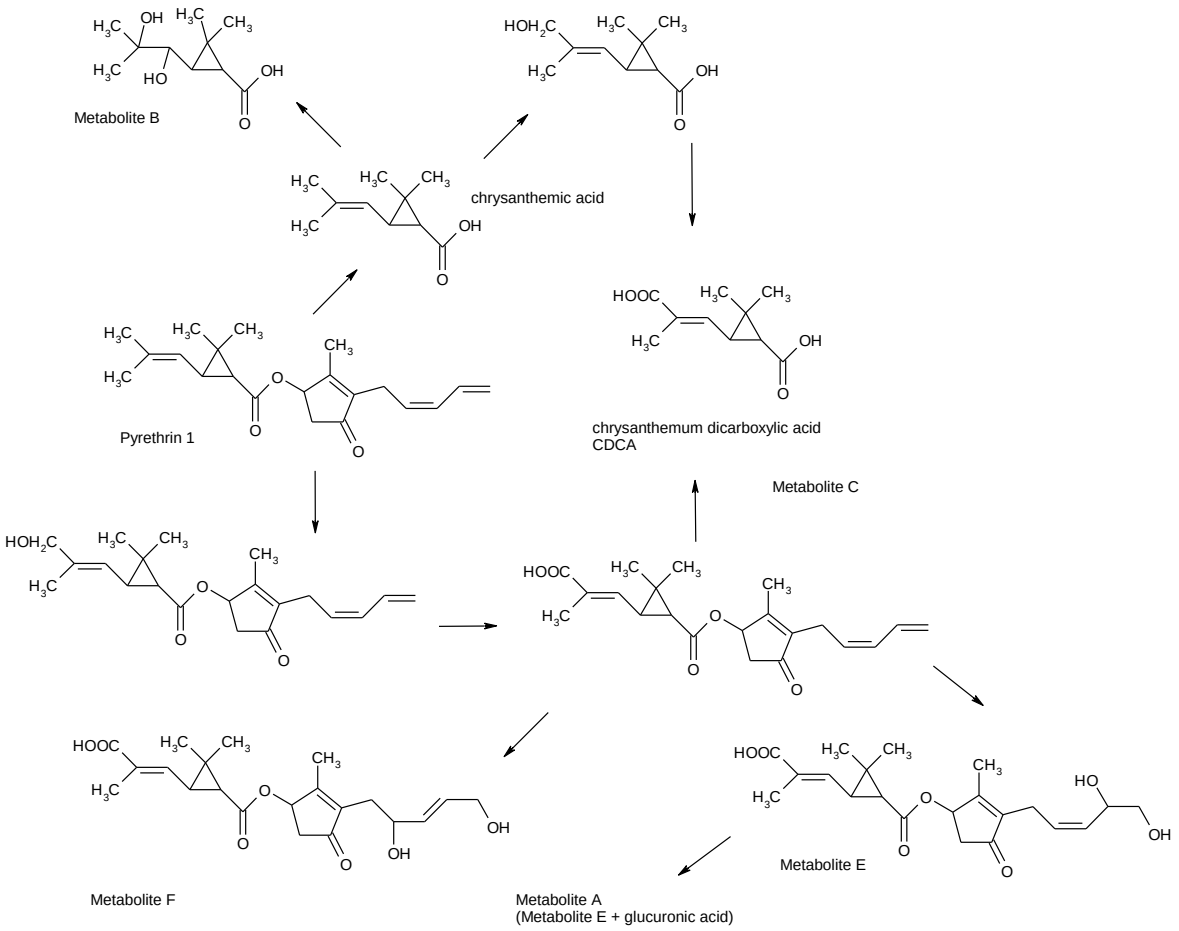


Figure 1: Proposed metabolic pathway for pyrethrin 1 in the rat

Dermal absorption

Two studies were provided.

[redacted] **A single dose, open label study to investigate the absorption and excretion of orally administered or dermally applied [^{14}C]-labelled pyrethrin I (PI) to healthy male volunteers.**

The clinical proportion of the study was conducted in compliance with "Declaration of Helsinki" (as revised in Edinburgh, Scotland, October 2000); the rules of good clinical practice U.S. FDA (protection of human subjects, 21 CFR, Ch. 1, Part 500; Institutional review boards, 21 CFR, Ch. 1, part 56; Investigational New Drug Application, 21 CFR, Ch.1, Part 312) and Rules of Good Clinical Practice European Community (Good clinical practice for trials on medicinal products in the European Community, III/3976/88-EN-Final).

The analytical part of the study was conducted in compliance with the FDA Good Laboratory Practice Regulation (GLP) as set forth in Title 21 of the U.S. Code of federal regulations, Part 58, issued December 22, 1978 (and all applicable revisions) and the applicable regulations of the OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17, OECD, Paris, 1998.

The study protocol was reviewed and approved by an Independent Ethics Committee and written informed consents were obtained by all eligible volunteers. The study is therefore

valid.

The fogger being tested contained pyrethrin as the active substance and co-formulants, the main of which is added to pyrethrin products to inhibit insects' microsomal enzymes that detoxify pyrethrins.

This study was conducted in 4 human male volunteers. The test material was applied to the volar aspect of the non-dominant forearm and the application site was covered with a non-occlusive aluminium dome, which allowed free movement of air but helped prevent loss of radioactivity due to physical contact with the application site, secured in place with an adhesive bandage. The test material remained in contact with the skin for approximately 8 hours. After exposure, the protective devices were removed, and the skin was wiped with cotton swabs dipped in 2% soapy water. The application site was dried with two cotton swabs, and then swabbed with two cotton swabs dipped in isopropyl alcohol (IPA), followed by four cotton swabs dipped in soapy water and four swabs dipped in IPA. One sixth of the dose site was also stripped with tape approximately 45 hours after the removal of test material. The purpose of the tape stripping was to determine the amount residual radioactivity associated with the surface layer of the skin. The skin site was also swabbed with cotton swabs dipped in IPA on Day 5. All urine and faeces excreted by the volunteers were collected for five days following dose administration. The protective enclosures, swabs, rinses, gauze, tape strips, urine and faeces were analysed for total radioactivity in order to determine mass balance and the percent of administered dose that was absorbed. In order to obtain information on the onset, rate and duration of absorption, venous blood samples were collected from the ipsilateral and contralateral veins during and after the 8-h exposure period; after separation, plasma samples were analysed for total radioactivity.

For the dermal dosing solution, MGK provided the testing facility with the blank of a typical fogger formulation containing all non-radiolabelled components but the active substance, similar to the commercial formulation. Radiolabelled pyrethrins I (PI) was added to the blank of the fogger formulation and mixed well to provide a homogeneously labelled formulation. The test substance formulation containing [^{14}C]-PI was administered dermally at approximately 50 µL of [^{14}C]-PI at a mean dose of 12.25 µg/cm^2 (the dose was administered over an area of 4x6 cm or 24 cm^2). The concentration of PI in the fogger formulation (dosing formulation) was 0.3 mg per 50 mg (0.6%). Each 50 mg of fogger formulation contained approximately 50 µCi of radioactivity. Volunteer identification, body weight and dose information for volunteers is shown below.

Volunteer Identification	**Volunteer Body weight (kg)**	**Total µCi Applied**	**Total µg PI Applied***	**Total PI Applied (µg/kg)**	**Dose formulation Administered (mg)**
01 ER	65.4	44.2	280	4.28	47.6
02 PR	81.7	44.6	282	3.45	48.0
03 CH	75.1	47.2	299	3.98	47.8
04 HB	91.3	49.6	314	3.44	50.3
Mean	**78.4**	**46.4**	**294**	**3.79**	**48.43**
± Standard deviation	10.91	2.51	15.97	0.41	1.26

* Calculated based on specific activity

In the dermally dosed subjects, no elevation in radioactivity was seen in the contralateral and ipsilateral plasma samples, reflecting the very slow dermal absorption of the radiolabelled PI. This was confirmed by the very low percent of dosed radioactivity (0.22%) that crossed the skin and was excreted in urine. Urinary excretion was rapid, with the highest percent of dosed radioactivity excreted between 12 and 24 hours. The overall mean recovery of radioactivity was 104.28%. The majority of the applied

radioactivity was accounted for on the surface of the skin, mainly the swabs, skin rinses, protective enclosure and gauze with a mean of 104.28% of the applied radioactivity. The first four soapy water dermal swabs removed a mean of 88.03% of the applied radioactivity. The remaining dermal swabs removed a mean of 12.06% of the applied radioactivity. Tape stripping and swabbing of 1/6 of the dosed area removed a mean of 0.06% of applied radioactivity, indicating there is no accumulation of radioactivity in the skin.

Since a good material balance was achieved and only 0.22% of the applied radioactivity was recovered in the excreta, the dermal absorption of PI was determined to be 0.22%. A summary of the data is shown in the tables below.

Time-course of urinary excretion Percent of applied radioactivity						
Time (hours)	Subject Number				Mean	Standard deviation
	01	02	03	04		
Pre-dose	0.00	0.00	0.00	0.00	0.00	0.00
0-4	0.00	0.00	0.01	0.02	0.01	0.01
4-8	0.04	0.03	0.04	0.06	0.04	0.01
8-12	0.05	0.04	0.04	0.06	0.05	0.01
12-24	0.09	0.09	0.06	0.08	0.08	0.01
24-36	0.08	0.08	0.00	0.00	0.04	0.05
36-48	0.02	0.00	0.00	0.00	0.01	0.01
48-60	0.00	0.00	0.00	0.00	0.00	0.00
60-72	0.00	0.00	0.00	0.00	0.00	0.00
72-84	0.00	0.00	0.00	0.00	0.00	0.00
84-96	0.00	0.00	0.00	0.00	0.00	0.00
96-120	0.00	0.00	0.00	0.00	0.00	0.00
Subtotal	0.28	0.24	0.15	0.22	0.22	0.05

Percent of applied radioactivity excreted in urine following dermal application of [^{14}C]-pyrethrin						
	Subject Number				Mean	Standard deviation
	01	02	03	04		
Subtotal	0.28	0.24	0.15	0.22	0.22	0.05
Percent of applied radioactivity excreted in faeces following dermal application of [^{14}C]-pyrethrin						
	Subject Number				Mean	Standard deviation
	01	02	03	04		
Subtotal	0.00	0.00	0.00	0.00	0.00	0.00
Percent of applied dose recovered in tape-stripping						
	Subject Number				Mean	Standard deviation
	01	02	03	04		
Subtotal	0.04	0.1	0.05	0.05	0.06	0.03
Percent of applied radioactivity recovered from skin surface						
	Subject Number				Mean	Standard deviation
	01	02	03	04		
Duoderm	0.74	0.61	0.32	0.82	0.62	0.22

Aluminium dome	0.48	0.74	1.61	0.44	0.82	0.54
Dermal swabs	104.06	100.05	98.71	97.55	100.09	2.84
Skin rinse	1.88	3.54	1.47	1.88	2.19	0.92
Gauze	0.30	0.45	0.16	0.18	0.27	0.13
Tape strips	0.04	0.10	0.05	0.05	0.06	0.03
Subtotal	107.5	105.49	102.32	100.92	104.06	2.99

Total recovery of radioactivity following dermal application of [^{14}C]-pyrethrin						
	Subject Number				Mean	Standard deviation
	01	02	03	04		
Surface radioactivity	107.5	105.49	102.32	100.92	104.06	2.99
Urine	0.28	0.24	0.15	0.22	0.22	0.05
Faeces	0.00	0.00	0.00	0.00	0.00	0.00
Total	**0.28**	**0.24**	**0.15**	**0.22**	**0.22**	**0.05**

[redacted] **Human *in vivo* Percutaneous absorption of pyrethrin and piperonyl butoxide. Fd Chem. Toxic. Vol. 32, No 1, pp. 51-53.**

This study was conducted in six male volunteers from whom informed consent was obtained. The [^{14}C] formulation, similar to the commercial formulation, was spread on the ventral forearm and after 30 minutes the application site was washed with soap and water. Urine was collected on day-1 in divided interval (0-4, 4-8, 8-12, 12-25 hours) and on days 2-7 as total daily excretions. The commercial formulation used contained 0.3% pyrethrin. [^{14}C]-pyrethrin (3.8 mCi/mmol) was applied at 5.25 µCi (487 µg) in a dosing volume of 200 µL spread over 88 cm^2 = 5.53 µg/cm^2. Percutaneous absorption was determined by ^{14}C urinary excretion, and the following formula: dose absorbed = (total ^{14}C urinary excretion of topical dose in man ÷ total ^{14}C urinary excretion of parenteral dose in monkey) x100. In monkeys, 22.5% of pyrethrins were excreted in urine following a parenteral dose. The percutaneous absorption of pyrethrin from the ventral forearm was calculated to be about 2% (1.9% ± 1.2), as shown in the table below.

Subject	Percentage absorbed
1	1.4
2	1.6
3	2.0
4	0.6
5	1.6
6	4.1
Mean ± SD	1.9 ± 1.2

A2.1.2 Values and conclusions used for the risk assessment

Value(s) used in the Risk Assessment – Oral absorption	
Value(s)	100%
Justification for the selected value(s)	Default absorption value of 100% used. Fast absorption occurred with >90% systematically absorbed in 5 to 8 hours ([redacted]).

Value(s) used in the Risk Assessment – Dermal absorption	
Value(s) **	0.3%

Justification for the selected value(s)	Evaluation of the Human volunteer dermal absorption studies highlighted several deficiencies in the study conducted by [redacted]. The clinical portion of the study was not conducted according to good clinical practice, and the analytical component of the study was not conducted in accordance with GLP standards. The exposure period of the study was just 30 minutes, and the study was not a mass-balance study. Instead, the dermal absorption values were determined by comparing urinary excretion values from the human volunteers with urinary excretion values obtained from monkeys parenterally administered pyrethrins (exact route not specified). No analytical data were reported. In contrast, the study conducted by [redacted] was conducted according to good clinical practice and GLP standards. The study was a mass-balance study that reported good recovery values with low variability between volunteers. The volunteers were dermally exposed for eight hours under controlled conditions and the analytical data are presented. As [redacted] was conducted in vivo in human volunteers, there should be no need to adjust the obtained dermal absorption value of 0.22%. However, as it cannot be excluded that the tested formulation differs from the commercial product(s) containing pyrethrins, results are re-evaluated in line with the EFSA guidance on dermal absorption (EFSA Journal 2017;15(6):4873) here below. In [redacted] absorption of pyrethrins is complete i.e. more than 75% excretion in urine occurs within half of the study period. The low residue removed by tape stripping can, therefore, be excluded for deriving the dermal absorption value. The preferred approach to addressing variability between replicates is to add a multiple of the standard deviation to the mean value. Although variability among the four volunteers is low (<25%), considering that 4 valid replicates are available, the multiplication factor is 1.6. Therefore, the final dermal absorption value for pyrethrins from biocidal product(s) is estimated to be 0.3% (i.e. 0.22 + (0.05 x1.6) %). A dermal absorption value of 0.3% is considered appropriate for the purposes of human health risk assessment for biocidal products containing pyrethrins as the active substance.

**The dermal absorption value is applicable for the active substance and might not be usable in product authorization.

Value(s) used in the Risk Assessment – Inhalatory absorption	
Value(s)	100%
Justification for the selected value(s)	Default worst-case value.

A.2.2. Acute toxicity / STOT SE

Oral Acute Toxicity

Independent from the ratio of Pyrethrins I and Pyrethrins II, Pyrethrum Extract exhibited low and moderate acute oral toxicity to male and female rats, respectively. Transient

clinical signs of toxicity in surviving animals were hyperactivity, ruffled fur and tremors ([redacted]). (KPIC)

Dose [mg/kg] Total pyrethrins/ Extract	**Number of dead / number of investigated**	**Time of death (range)**	**Observations**
229	-	-	
355	-	-	
560	-	-	
710	-	-	
794	-	-	
891/1365	0/5	-	All animals appeared normal throughout the 14-day observation period.
1410/2160	0/5	-	In the beginning of the test period one animal exhibited dark nasal staining. All the others appeared normal until the end of the test phase.
2230/3417	2/5	day 1	In the beginning appearance of ruffled fur and tremors. After 1 day two animals were found dead, after the second day the remaining animals appeared normal.
2660/4075	4/5	day 1	Initially 2 of 5 animals exhibited tremors, after 1 day four animals were found dead.
3550/5439	5/5	day 1	After one day all animals found dead without any clinical signs after immediately treatment and 4 h later on.
LD_{50} value	**2140 mg/kg bw (1730 - 2640 mg/kg bw) total pyrethrins; 3269 mg/kg bw (2651 - 4045 mg/kg bw) extract**		

Dose [mg/kg] Total pyrethrins/ Extract	**Number of dead / number of investigated**	**Time of death (range)**	**Observations**
229/351	0/5	-	All animals appeared normal throughout the 14-day observation period.
355/544	1/5	1 day	After 1 day one rat was found dead, whereas all the other animals appeared normal throughout the observation period.
560/858	1/5	1 day	After 4 h all animals exhibited tremors, one day later three of them appeared normal, one was found dead and one exhibited dark nasal and and ocular staining.
710/1088	1/5	4 h	After 4 h one animal was found dead, the other exhibited tremors. After one day all animals appeared normal throughout the test period.
794/1217	3/5	4 h-1 day	After 2 h three animals exhibited tremors, after 4 h one was found dead. Two other rats were found dead after 1 day, the rest appeared normal throughout the test period.
891/1365	5/5	4 h-1 day	After 4 h three of the test animals were already found dead, the rest have died one day after treatment.
1410	-	-	
2230	-	-	
2660	-	-	
3550	-	-	
LD_{50} value	**700 mg/kg bw (500 – 990 mg/kg bw) total pyrethrins; 1073 mg/kg bw (766 – 1517 mg/kg bw) extract**		

Oral acute toxicity study was conducted in Sprague-Dawley rats. Pyrethrum extract was dosed to 6 groups of males (5/group) at concentrations between 710 and 5000 mg/kg bw total pyrethrins (1088 and 7661 mg/kg bw extract) and 7 groups of females (5/group) at concentration between 316 and 2000 mg/kg bw total pyrethrins (484 and 3064 mg/kg bw extract). In males, no deaths were recorded at the 710 and 891 mg/kg bw total pyrethrins (1088 and 1365 mg/kg bw extract) dose levels. There were 2 deaths at the 2230 mg/kg bw total pyrethrins (3417 mg/kg bw extract) dose level, 3 deaths at the 2660 mg/kg bw total pyrethrins (4076 mg/kg bw extract) dose level, 4 deaths at the 3550 mg/kg bw total pyrethrins (5439 mg/kg bw extract) dose level and 5 deaths at the 5000 mg/kg bw total pyrethrins (7661 mg/kg bw extract) dose level. In females, no deaths were recorded at the 316, 500 and 794 mg/kg bw total pyrethrins (484, 766, and 1217 mg/kg bw extract) dose levels. There were 2 deaths at the 944 mg/kg bw total pyrethrins (1446 mg/kg bw extract) dose level, 3 deaths at the 1120 mg/kg bw total pyrethrins (1716 mg/kg bw extract) dose level and 5 deaths at the 1260 and 2000 mg/kg bw total pyrethrins (1931 and 3064 mg/kg extract) dose levels. Clinical signs were observed in male rats at a dose level greater than 710 mg/kg bw total pyrethrins (1088 mg/kg bw extract) (ruffling and tremors) and in female groups at a dose level greater than 316 mg/kg bw total pyrethrins (484 mg/kg bw extract) (tremors and hyperactivity). All surviving animals gained weight throughout the study. At necropsy, no internal abnormalities were observed at doses of 891 mg/kg bw total pyrethrins (1365 mg/kg bw extract) or less for males and 794 mg/kg bw total pyrethrins (1217 mg/kg bw extract) or less for females. At doses higher than 891/794 mg/kg bw total pyrethrins (1365/1217 mg/kg bw extract) male/female observations such as muzzle staining, coloured material in the lower gastrointestinal tract and haemorrhagic lungs were noted. The oral LD_{50} of Pyrethrum extract was found to be 2370 (1680 - 3350) mg/kg bw total

pyrethrins (3631 (2574 - 5133) mg/kg bw extract) for males and 1030 (860 - 1240) mg/kg bw total pyrehtrins (1578 (1318 - 1900) mg/kg bw extract) for females. The no effect level for clinical signs was 710 mg/kg bw total pyrethrins (1088 mg/kg bw extract) in males and 316 mg/kg bw total pyrethrins (484 mg/kg bw extract) in females ([redacted]). (BRA, MGK and SCJ)

Mortality data					
Dose (mg/kg bw) Total pyrethrins/ Extract	**Males Mortality**	**Time of death – days (no of animals)**	**Dose (mg/kg bw) Total pyrethrins/ Extract**	**Females Mortality**	**Time of death - days (no of animals)**
710/1088	0/5	-	316/484	0/5	-
891/1365	0/5	-	500/766	0/5	-
2230/3417	2/5	1 (2)	794/1217	0/5	-
2660/4076	3/5	1 (3)	944/1446	2/5	1 (2)
3550/5439	4/5	1 (3), 2 (1)	1120/1716	3/5	1 (3)
5000/7661	5/5	1(5)	1260/1931	5/5	0 (1), 1 (4)
-	-	-	2000/3064	5/5	0 (1), 1 (4)
LD_{50} value	2370 mg/kg bw total pyrethrins 3631 mg/kg bw extract		1030 mg/kg bw total pyrethrins 1578 mg/kg bw extract		

Dermal Acute Toxicity

The acute dermal toxicity of Pyrethrum extract was investigated in rabbits. A single dose of active ingredient of Pyrethrum extract was applied to the skin of 5 New Zealand White rabbit/sex at a concentration of 2000 mg/kg bw total pyrethrins (3064 mg/kg bw extract) using an occlusive material. After a 24-hour dermal exposure period to Pyrethrum extract, male and female rabbits presented very slight to well defined erythema, slight oedema and a stained test site. All males appeared normal after 6 days and all females after 9 days. All animals gained weight during the study. No internal abnormalities were observed at gross necropsy in any animal. The percutaneous LD_{50} of Pyrethrum extract was found to be in excess of 2000 mg/kg bw total pyrethrins (3064 mg/kg bw extract) ([redacted]). (KPIC and BRA, MGK and SCJ)

Dose (mg/kg)	**Males Mortality**	**Time of death – days (no of animals)**	**Dose (mg/kg)**	**Females Mortality**	**Time of death - days (no of animals)**
2000	0/5	-	2000	0/5	-
LD_{50} value	> 2000 mg/kg bw total pyrethrins >3064 mg/kg bw extract		LD_{50} value	> 2000 mg/kg bw total pyrethrins >3064 mg/kg bw extract	

Inhalation Acute Toxicity

In order to assess the acute inhalation toxicity, 4 groups of 5 Sprague-Dawley rats/sex/group were exposed to Pyrethrum extract for 4 hours at the following analytical concentrations: 0 (acetone control), 0.69, 2.1 and 4.6 mg/L total pyrethrum (0, 1.06, 3.2, and 7.1 mg/L extract). Pyrethrum extract was administered as a liquid aerosol by the inhalation route, using acetone as a vehicle. In males, no deaths were recorded at 0, 0.69 and 2.1 mg/L total pyrethrins (0, 1.06, and 3.2 mg/L extract) dose levels. There were 2 deaths at 4.6 mg/L total pyrethrins (7.1 mg/L exgract) dose level. In females, there were no deaths recorded at 0 and 0.69 mg/L total pyrethrins (0 and 1.06 mg/L extract) dose levels. 2 rats died at 2.1 mg/L total pyrethrins (3.2 mg/L extract) dose level and 4 rats died at 4.6 mg/L total pyrethrins (7.1 mg/L extract) dose level. During exposure the animals exhibited laboured breathing, excessive salivation, decreased activity and eye closure. Among the acetone-control group, similar effects were observed with an additional excessive lacrimation and nasal discharge. However, tremors were also noted during the higher-level exposures. These observations were noted

immediately following exposure but after several days the signs decreased significantly. All groups of animals lost weight on the day following treatment. However, recovery of weight occurred over time and all surviving animals were in excess of weight at the end of the study. Reddening of the lung and turbinates and lung oedema were the major post-mortem findings which were considered to be related to exposure to Pyrethrum extract. The LC_{50} was calculated to be 3.4 mg/L total pyrethrins (5.2 mg/L extract) for the combined sexes (2.3-4.9) ([redacted]). (KPIC and BRA, MGK and SCJ)

Group	Concentration (mg/L)		Mortality			LC_{50} (14d)
	Nominal	Analytical	Males	Females	Total	Dose (mg/L) Total pyrethrins/ Extract
I	14	4.6 ± 0.5[1)]	3/5	4/5	7/10	3.4 (2.3-4.9) 5.2 (3.5-7.5) [3)]
II	-[2)]	-	0/5	0/5	0/10	
III	5.6	2.1 ± 0.4	0/5	2/5	2/10	
IV	0.92	0.69 ± 0.08	0/5	0/5	0/10	

[1)] mean ± standard deviation
[2)] vehicle control
[3)] 95 % confidence limits

A.2.2.1. *Acute oral toxicity*

Table A.11 Summary table of animal studies on acute oral toxicity

Summary table of animal studies on acute oral toxicity						
Method, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity) Vehicle, Dose levels, Type of administration (gavage, in diet, other)	Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)	Value LD_{50}	Remarks (e.g. major deviations)	Reference
US EPA 81-1, OECD 401 GLP Reliability 1 Key	Rat Sprague-Dawley male + female 5 per dose/sex	Pyrethrum Extract (FEK-99; 57.574%) Male: 891, 1410, 2230, 1660, and 3550 mg/kg bw total pyrethrins; 1365, 2160, 3417, 4075, and 5439 mg/kg bw extract Female: 229, 355, 560, 710, 794, and 891 mg/kg bw total pyrethrins; 351, 544, 858, 1088, 1217, and 1365 mg/kg bw extract Single dose (gavage)	Transient clinical signs of toxicity in surviving animals were hyperactivity, ruffled fur and tremors.	Male: 2140 mg/kg bw (1730-2640 mg/kg bw) total pyrethrins; 3269 mg/kg bw (2651-4045 mg/kg bw) extract Female:700 mg/kg bw (500-990 mg/kg bw) total pyrethrins; 1073 mg/kg bw (766-1517 mg/kg bw) extract	-	[redacted] (KPIC) IIIA6.1.1

EPA OPP 81-1 and OECD Guideline 401 GLP Reliability 1 Key	Rat Sprague-Dawley Male/Female 5/sex/group	Pyrethrum extract (FEK-99; 57.03%) Males:710, 891, 2230, 2660, 3550, and 5000 mg/kg total pyrethrins; 1088, 1365, 3417, 4076, 5439, and 7661 mg/kg bw extract Females: 316, 500, 794, 944, 1120, 1260, and 2000 mg/kg total pyrethrins; 484, 766, 1217, 1446, 1716, 1931, and 3064 mg/kg bw extract Single dose (gavage)	Males >710 mg/kg bw total pyrethrins (1088 mg/kg bw extract): rufflings and tremors Females >316 mg/kg bw total pyrethrins (484 mg/kg bw extract): tremors and hyperactivity. Females >794 and Males >891 mg/kg bw (1217 and 1365 mg/kg bw extract respectively): muzzle staining, coloured material in the lower gastrointestinal tract and haemorrhagic lungs	Males: 2370 (1680 - 3350) mg/kg bw total pyrethrins; 3631 (2574 - 5133) mg/kg bw extract Females: 1030 (860 - 1240) mg/kg bw total pyrethrins; 1578 (1318 - 1900) mg/kg bw extract		[redacted] (BRA, MGK and SCJ) IIIA 6.1.1

Table A.12 Summary table of human data on acute oral toxicity

No data are available.

Table A.13 Summary table of other studies relevant for acute oral toxicity

No data are available.

A2.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Chrysanthemum cinerariaefolium extract from HCS exhibited low and moderate acute oral toxicity to male and female rats, respectively.

A2.2.1.2 Comparison with the CLP criteria

Classified as Acute Tox. 4, (H302: Harmful if swallowed) because, according to the CLP regulation 3.1.2.1. (Table 3.1.1), 300 mg/kg bw < LD_{50} ≤ 2000 mg/kg bw.

A2.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Acute toxicity Category 4, H302: Harmful if swallowed. ATE oral = 700 mg/kg bw total pyrethrins; ATE oral = 1073 mg/kg bw extract.

A2.2.1.4 Conclusion on acute oral toxicity related to risk assessment

Value used in the Risk Assessment – Acute oral toxicity	
Value	700 mg/kg bw total pyrethrins; 1073 mg/kg bw extract. Acute Tox. 4, H302: Harmful if swallowed
Justification for the selected value	Lowest LD_{50} value

A.2.2.2. *Acute dermal toxicity*

Table A.14 Summary table of animal studies on acute dermal toxicity

Summary table of animal studies on acute dermal toxicity						
Method, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity), Vehicle, Dose levels, Surface area	Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)	Value LD_{50}	Remarks (e.g. major deviations)	Reference
EPA OPP 81-2 and OECD Guideline 402 GLP Reliability 1 Key	Rabbit New Zealand White Male/Female 5/sex/group	Pyrethrum Extract (FEK-99; 57.467%) 2000 mg/kg bw total pyrethrins; 3064 mg/kg bw extract Occlusive 24 h	No systemic effects. Males and females: slight-well defined erythema, slight edema and stained test site. Recovering after several days.	>2000 mg/kg bw total pyrethrins; >3064 mg/kg bw extract	-	[redacted] IIIA6.1.2 (KPIC) (BRA, MGK and SCJ)

Table A.15 Summary table of human data on acute dermal toxicity

No data are available.

Table A.20 Summary table of other studies relevant for acute dermal toxicity

No data are available

A2.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Dermal application of 2000 mg/kg bw total pyrethrins (3064 mg/kg bw extract) did not cause death or systemic clinical signs of toxicity.

A2.2.2.2 Comparison with the CLP criteria

Not classified because, according to the CLP regulation 3.1.2.1. (Table 3.1.1), LD_{50} > 2000 mg/kg bw.

A2.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Substance does not meet the criteria to be classified in this hazard class.

A2.2.2.4 Conclusion on acute dermal toxicity related to risk assessment

Value used in the Risk Assessment – Acute dermal toxicity	
Value	>2000 mg/kg bw total pyrethrins; >3064 mg/kg bw extract
Justification for the selected value	Highest dose tested.

A.2.2.3. *Acute inhalation toxicity*

Table A.21 Summary table of animal studies on acute inhalation toxicity

Summary table of animal studies on acute inhalation toxicity						
Method, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD) Actual and nominal concentration, Type of administration (nose only / whole body/ head only)	Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)	Value LC_{50}	Remarks (e.g. major deviations)	Reference
US EPA 81-3, OECD 403 GLP Reliability 1 Key	Rat Sprague-Dawley male + female 5 per dose/sex	Pyrethrum Extract (FEK-99; 57.574%) MMAD = 2.6 µm ± 2.2 µm 0, 0.69, 2.1, and 4.6 mg/L total pyrethrins; 0, 1.06, 3.2, and 7.1 mg/L extract analytical concentration 4 h – Whole body	Post-mortem findings were: reddening of the lung and turbinates and lung oedema.	LC_{50} males: 3.9 mg/L (2.1-7.2 mg/L with 95% confidence limit) total pyrethrins; 6.0 mg/L (3.2-11.0 mg/L with 95% confidence limit) extract LC_{50} females: 2.5 mg/L (1.5-4.3 mg/L with 95% confidence limit) total pyrethrins; 3.8 mg/L (2.3-6.6 mg/L with 95% confidence limit) extract LC_{50} both sexes: 3.4 mg/L (2.3-4.9 mg/L with 95% confidence limit) total pyrethrins; 5.2 mg/L (3.5-7.5 mg/L with 95% confidence limit) extract	-	[redacted] (KPIC) (BRA, MGK and SCJ) IIIA6.1.3

Table A.16 Summary table of human data on acute inhalation toxicity
No data are available.

Table A.17 Summary table of other studies relevant for acute inhalation toxicity

No data are available.

A2.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Inhalation exposure to different concentrations of Pyrethrum Extract as an aerosol to rats resulted in a combined LC_{50} (both sexes) of 3.4 mg/L total pyrethrins (5.2 mg/L extract).

A2.2.3.2 Comparison with the CLP criteria

Classified as Category 4: Acute Tox. 4 (H332: Harmful if inhaled) because, according to the CLP 3.1.2.1. (Table 3.1.1), 1.0 mg/L < LC_{50} ≤ 5.0 mg/L according to the toxicity test result.

A2.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Acute toxicity Category 4 H332: Harmful if inhaled. ATE inhalation = 2.5 mg/L total pyrethrins (dusts and mists). ATE inhalation = 3.8 mg/L extract (dusts and mists).

A2.2.3.4 Conclusion on acute inhalation toxicity related to risk assessment

Value used in the Risk Assessment – Acute inhalation toxicity	
Value	2.5 mg/L total pyrethrins. 3.8 mg/L extract. Acute Tox. 4, H332: Harmful if inhaled.
Justification for the selected value	Lowest LC_{50} value.

A.2.2.4. *Specific target organ toxicity – single exposure Category 1 and 2 (STOT SE 1 and 2)*

A2.2.4.1 Short summary and overall relevance of the provided information on STOT SE 1 and 2

Effects observed in oral acute toxicity study were in males, no deaths at the 710 and 891 mg/kg bw total pyrethrins (1088 and 1365 mg/kg bw extract) dose levels. There were 2 deaths at the 2230 mg/kg bw total pyrethrins (3417 mg/kg bw extract) dose level, 3 deaths at the 2660 mg/kg bw total pyrethrins (4076 mg/kg bw extract) dose level, 4 deaths at the 3550 mg/kg bw total pyrethrins (5439 mg/kg bw extract) dose level and 5 deaths at the 5000 mg/kg bw total pyrethrins (7661 mg/kg bw extract) dose level. In females, no deaths were recorded at the 316, 500 and 794 mg/kg bw total pyrethrins (484, 766 and 1217 mg/kg bw extract) dose levels. There were 2 deaths at the 944 mg/kg bw total pyrethrins (1446 mg/kg bw extract) dose level, 3 deaths at the 1120 mg/kg bw total pyrethrins (1716 mg/kg bw extract) dose level and 5 deaths at the 1260 and 2000 mg/kg bw total pyrethrins (1931 and 3064 mg/kg bw extract) dose levels. Clinical signs were observed in male rats at a dose level greater than 710 mg/kg bw total pyrethrins (1088 mg/kg bw extract) (ruffling and tremors) and in female groups at a dose level greater than 316 mg/kg bw total pyrethrins (484 mg/kg bw extract) (tremors and hyperactivity). All surviving animals gained weight throughout the study. At necropsy, no internal abnormalities were observed at doses of 891 mg/kg bw total pyrethrins (1365 mg/kg bw extract) or less for males and 794 mg/kg bw total pyrethrins (1217 mg/kg bw extract) or less for females. At doses higher than 891/794 mg/kg bw total pyrethrins (1365/1217 mg/kg bw extract) male/female observations such as

muzzle staining, coloured material in the lower gastrointestinal tract and haemorrhagic lungs were noted. The oral LD_{50} of Pyrethrum extract was found to be 2370 (1680 - 3350) mg/kg bw total pyrethrins (3631 (2574 - 5133) mg/kg bw extract) for males and 1030 (860 - 1240) mg/kg bw total pyrethrins (1578 (1318 - 1900) mg/kg bw extract) for females. The no effect level for clinical signs was 710 mg/kg bw total pyrethrins (1088 mg/kg bw extract) in males and 316 mg/kg bw total pyrethrins (484 mg/kg bw extract) in females ([redacted]). (BRA, MGK and SCJ)

Effects observed in dermal acute toxicity study were very slight to well defined erythema, slight oedema and a stained test site in males and females. All males appeared normal after 6 days and all females after 9 days. All animals gained weight during the study. No internal abnormalities were observed at gross necropsy in any animal. The percutaneous LD50 of Pyrethrum extract was found to be in excess of 2000 mg/kg bw total pyrethrins (3064 mg/kg bw extract) ([redacted]). (BRA, MGK and SCJ)

Effects observed in inhalation acute toxicity study were in males, no deaths at 0, 0.69 and 2.1 mg/L total pyrethrins (0, 1.06 and 3.2 mg/L extract) dose levels. There were 2 deaths at 4.6 mg/L (7.1 mg/L extract) dose level. In females, there were no deaths recorded at 0 and 0.69 mg/L total pyrethrins (0 and 1.06 mg/L extract) dose levels. 2 rats died at 2.1 mg/L total pyrethrins (3.2 mg/L extract) dose level and 4 rats died at 4.6 mg/L total pyrethrins (7.1 mg/L extract) dose level. During exposure the animals exhibited laboured breathing, excessive salivation, decreased activity and eye closure. Among the acetone-control group, similar effects were observed with an additional excessive lacrimation and nasal discharge. However, tremors were also noted during the higher-level exposures. These observations were noted immediately following exposure but after several days the signs decreased significantly. All groups of animals lost weight on the day following treatment. However, recovery of weight occurred over time and all surviving animals were in excess of weight at the end of the study. Reddening of the lung and turbinates and lung oedema were the major post-mortem findings which were considered to be related to exposure to Pyrethrum extract. The LC_{50} was calculated to be 3.4 mg/L total pyrethrins (5.2 mg/L extract) for the combined sexes (2.3-4.9 mg/L total pyrethrins (3.5-7.5 mg/L extract)) ([redacted]). (BRA, MGK and SCJ)

Regarding the effects observed in the oral, dermal and inhalation toxicity studies, all of them are not organ-specific. Indeed the specific findings are those typical of the route of exposure (effects in the gastrointestinal or respiratory tracts or in the skin) or related with systemic toxicity.

In a neurotoxicity study, groups of 15 male Sprague-Dawley rats received by gavage a 10% w/v solution of Pyrethrum extract in corn oil at doses of 0, 40, 125 or 400 mg/kg bw total pyrethrins (0, 61, 192, and 613 mg/kg bw extract), and 15 females received a 5% w/v solution of Pyrethrum extract in corn oil at doses of 0, 20, 63 or 200 mg/kg bw total pyrethrins (0, 31, 97, and 306 mg/kg bw extract).

Five males and two females at the high dose died on the day of treatment, and a variety of acute neurological signs were observed in the other animals at this dose, including tremors, urogenital area wetness, salivation, perinasal encrustation, exaggerated startle response, decreased grip strength, hind leg splay, and increased body temperature. Tremors were also observed in three females at the intermediate dose. Measurements of motor activity on the day of treatment indicated increased fine movement and decreased rearing and ambulation in animals of each sex at the high dose and decreased fine movement, rearing and ambulation in males as the intermediate dose. This is likely due to an effect of treatment and a predisposition for lower activity of this group if compared to the control group during pre-treatment evaluation.

In addition, slight, statistically non-significant decreases in body weight were seen in

males at the high dose on days 7 and 14. There was no evidence of any gross, treatment-related lesion. The microscopic changes were limited mainly to sections of the sciatic nerve and its branches. The histomorphological changes within the peripheral nerve sections indicated the presence of scattered degenerating nerve fibres or myelin sheaths. These changes were seen in only a few animals, were graded as minimal, and were not dose-related.

Males		**Females**	
Dosage (mg/kg total pyrethrins (mg/kg bw extract))	**Number surviving /number initiated**	**Dosage (mg/kg total pyrethrins (mg/kg bw extract))**	**Number surviving /number initiated**
0 (0)	15/15	0 (0)	15/15
40 (61)	15/15	20 (31)	15/15
125 (192)	15/15	63 (97)	15/15
400 (613)	10/15	200 (306)	13/15

Time point	**0 mg/kg bw total pyrethrins 0 mg/kg bw extract**	**40 mg/kg bw total pyrethrins 61 mg/kg bw extract**	**125 mg/kg bw total pyrethrins 192 mg/kg bw extract**	**400 mg/kg bw total pyrethrins 613 mg/kg bw extract**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
0 hour	200.47 **±** 11.259	201.07 **±** 12.411	199.26 **±** 10.176	195.81 **±** 11.173
3 hours	239.88 **±** 15.132	239.99 **±** 14.168	238.98 ± 11.009	230.40 **±** 14.976
7 days	281.94 **±** 18.795	282.53 **±** 20.391	281.99 **±** 16.150	269.27 **±** 19.463
14 days	320.53 **±** 23.108	319.69 **±** 24.069	318.91 **±** 20.345	304.24 **±** 27.035

S.D. – Standard deviation
0 hour – Pretreatment

Time point	**0 mg/kg bw total pyrethrins 0 mg/kg bw extract**	**20 mg/kg bw total pyrethrins 31 mg/kg bw extract**	**63 mg/kg bw total pyrethrins 97 mg/kg bw extract**	**200 mg/kg bw total pyrethrins 306 mg/kg bw extract**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
0 hour	146.30 **±** 8.146	144.67 **±** 10.838	142.98 **±** 8.952	146.52 **±** 7.365
3 hours	162.73 **±** 9.563	162.22 **±** 11.541	158.7 **±** 9.985	162.02 **±** 7.818
7 days	182.91 **±** 11.666	181.95 **±** 13.632	177.99 **±** 14.047	179.8 **±** 8.081
14 days	198.57 **±** 15.492	200.49 **±** 17.886	193.51 **±** 17.053	194.94 **±** 10.208

S.D. – Standard deviation
0 hour – Pretreatment

FEEDING LEVEL (mg/kg bw total pyrethrins (mg/kg bw extract))	**0 (0)**	**40 (61)**	**125 (192)**	**400 (613)**	**0 (0)**	**20 (31)**	**63 (97)**	**200 (306)**
	MALES				**FEMALES**			
NUMBER IN GROUP	15	15	15	15	15	15	15	15
SCIATIC NERVE								
myelin degeneration/myelin sheath swelling	0	0	0	2	0	0	0	1
minimal focal	0	0	0	2	0	0	0	1

myelin/axon degeneration	0	2	1	1	0	0	0	4
minimal focal	0	2	1	1	-	-	-	-
minimal multifocal	0	1	1	1	0	0	0	3
moderate multifocal	0	1	0	0	0	0	0	1
NERVE PERONEAL								
myelin/axon degeneration	-	-	-	-	0	1	0	2
minimal multifocal	-	-	-	-	0	1	0	1
moderate multifocal	-	-	-	-	0	0	0	1
NERVE TIBIAL								
myelin/axon degeneration	-	-	-	-	0	0	0	2
minimal multifocal	-	-	-	-	0	0	0	1
moderate multifocal	-	-	-	-	0	0	0	1

The NOEL/NOAEL was 40 mg/kg bw total pyrethrins (61 mg/kg bw extract) for male rats and 20 mg/kg bw total pyrethrins (31 mg/kg bw extract) for female rats ([redacted]). (KPIC and BRA, MGK and SCJ)

Taking into account that the observed effects are not specific for any organ, active substance does not classify for this hazard class.

A2.2.4.2 Comparison with the CLP criteria

No data are available to indicate that the active substance should be classified for STOT SE 1 or 2. Observed effects are not organ-specific and do not compromise the normal function of any organ as stated in CLP 3.8.2.1.7.3.

A2.2.4.3 Conclusion on classification and labelling for STOT SE 1 and 2

Chrysanthemum cinerariaefolium extract from HCS does not meet the EU criteria to be classified as STOT SE 1 or 2.

A.2.2.5. *Specific target organ toxicity – single exposure Category 3 (STOT SE 3)*

A2.2.5.1 Short summary and overall relevance of the provided information on STOT SE 3

Not classified.

A2.2.5.2 Comparison with the CLP criteria

No data are available to indicate that the active substance should be classified for STOT SE 3, H335: May cause respiratory irritation. Acute inhalation toxicity studies in animals are not enough to support this hazard class regarding CLP 3.8.2.2.1.d).

No data are available to indicate that the active substance should be classified for STOT SE 3, H336: May cause drowsiness and dizziness. Observed effects in the acute neurotoxicity study are treatment-related but they are strongly related with the behaviour noted in the pre-treatment phase. In some cases there are not a dose-response relationship.

A2.2.5.3 Conclusion on classification and labelling for STOT SE 3

Chrysanthemum cinerariaefolium extract from HCS does not meet the EU criteria to be classified as STOT SE 3.

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

Summary of the Dossier Submitter's proposal

STOT SE 1/STOT SE 2

The Dossier Submitter considered that the effects observed in the oral (including acute neurotoxicity study), dermal and inhalation toxicity studies, are not organ-specific, but rather typical for the route of exposure (effects in the gastrointestinal or respiratory tracts or in the skin) or related to systemic toxicity. These effects do not compromise the normal function of any organ.

Therefore, the Dossier Submitter concluded that *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent does not meet the CLP criteria for STOT SE 1 or 2.

STOT SE 3

The Dossier Submitter considered that no data are available to indicate that the active substance should be classified for STOT SE 3, H335: May cause respiratory irritation. Acute inhalation toxicity studies in animals are not enough to support this hazard class regarding CLP 3.8.2.2.1.d.

Also, no data are available to indicate that the active substance should be classified for STOT SE 3, H336: May cause drowsiness and dizziness. Observed effects in the acute neurotoxicity study were treatment-related (decreased fine movement, rearing and ambulation), but they are considered to be due to a predisposition for lower activity of animals in the treated group compared to the control group, observed already during pre-treatment evaluation.

Therefore, the Dossier Submitter concluded that *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent does not meet the CLP criteria for STOT SE 3.

Comments received during consultation

During the consultation for *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent, one MSCA suggested, referring to the RAR (2021), a classification as STOT SE 1;H370 with the nervous system as the target organ because neurotoxic symptoms occurred below the doses warranting the acute oral toxicity classification. The MSCA pointed out that according to the CLP Regulation and ECHA CLP Guidance, significant toxicity can be manifested as functional disturbance which was seen in the studies (neurobehavioural effects). In their response, the Dossier Submitter did not agree with the proposed classification and considered that the observed effects were neither significant nor severe and furthermore were transient.

During the later targeted consultation in 2023, one MSCA supported STOT SE 1 or 2

classification for the effects on nervous system.

Additional key elements

The tables below are taken from the Applicant's response to EFSA's request to perform a benchmark dose (BMD) analysis of laryngeal metaplasia incidences in rats following inhalation exposure to pyrethrins (within the procedure for renewal of the approval of active substances, i.e. Pyrethrins). The severity of the changes is graded as follows:

1 – minimal (affected epithelium was approximately 2-3 cell layers thick);
2 – slight (affected epithelium was approximately 4-5 cell layers thick);
3 – moderate (affected epithelium was approximately 6-7 cell layers thick);
4 – moderately severe (affected epithelium was approximately 8-10 cell layers thick).

Incidence and severity of squamous/squamoid metaplasia/hyperplasia in the larynx mucosa pseudostratified columnar epithelium reported in 90-day inhalation toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1992):

MALES					
Dose (mg/m3)	**0**	**11**	**30**	**100**	**356**
N of animals	14	15	15	15	15
Severity:					
1	2	0	1	2	0
2	0	7	4	5	0
3	0	3	8	3	9
4	0	0	0	5	6
Total	**2**	**10**	**13**	**15**	**15**
FEMALES					
Dose (mg/m3)	**0**	**11**	**30**	**100**	**356**
N of animals	15	14	15	15	15
Severity:					
1	0	2	0	0	0
2	0	9	7	4	3
3	0	2	5	6	10
4	0	0	0	1	2
Total	**0**	**13**	**12**	**11**	**15**

Incidence and severity of squamous/squamoid metaplasia/hyperplasia in the larynx ventral diverticulum cuboidal/columnar epithelium reported in 90-day inhalation toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1992):

MALES					
Dose (mg/m3)	**0**	**11**	**30**	**100**	**356**
N of animals	14	15	15	15	15
Severity:					
1	0	1	1	5	4
2	0	1	0	0	6
3	0	0	0	0	2
Total	**0**	**2**	**1**	**5**	**12**
FEMALES					
Dose (mg/m3)	**0**	**11**	**30**	**100**	**356**
N of animals	15	14	15	15	15
Severity:					
1	0	0	1	2	7
2	0	0	0	0	1
3	0	0	0	0	0
Total	**0**	**0**	**1**	**2**	**8**

Assessment and comparison with the classification criteria

Neurotoxicity

Pyrethrins exert acute neurotoxic effects of type I pyrethroids for which the mode of action is known, i.e. modification of voltage-sensitive sodium channels with delayed closure and protracted sodium influx. Clinical signs of neurotoxicity, characterised by fine tremor progressing to whole body tremor and prostration, paresthesia, aggressive sparring, increased sensitivity to external stimuli, enhanced salivation, and hunched posture, were observed in many acute and repeated-dose studies, and acute neurotoxicity of pyrethrins was specifically evaluated in the study described below.

Acute oral neurotoxicity study with pyrethrum extract in rats (KPIC/BRA, MGK, and SCJ, 1993*)*

This is a GLP-compliant study, performed according to OECD TG 424. Sprague-Dawley rats, 15 animals/sex/group, were treated once by oral gavage with the test substance

(FEK-99 blend; purity: 57.467%), as 10% solution (males) or 5% solution (females) in corn oil, at dose levels of 0, 42, 131, and 418 mg total pyrethrins/kg bw in males, and 0, 21, 66, and 209 mg total pyrethrins/kg bw in females (doses were selected on the basis of occurrence of mild-to-severe tremors in a dose-range finder study).

Methodology

Observations and body weight: All animals were observed for mortality twice each day, 7 days a week until sacrificed. Detailed clinical observations and body weight measurement were performed weekly untic sacrifice.

Neurobehavioral evaluations (FOB) were performed during the week prior to dosing and approximately 3 hours, 7 days, and 14 days following dosing. Testing at each measurement period was conducted in 6 replicates of 7 or 8 animals/sex.
Following endpoints were assessed: posture, tremor, unusual behaviour, breathing patter, urination, startle response, muscle tone, salivation, dehydration, visual placing, convulsions, vocalisation, gait, arousal, rears, tail pinch response, piloerection, exophtalmus, fur appearance, grip strength, air righting reflex, handling reactivity, palpebral closure, body position, defecation, approach response, pupil size, lacrimation, emaciation, crust (mouth, nose, or eyes), rectal temperature, and hindlimb splay.

Motor activity: Animals were tested using an automated recording apparatus designed to measure activity (fine movement, rearing and ambulation) in a novel environment. Motor activity was measured before the treatment and at 4 hour, 7 d and 14 d of the treatment period in all dose groups.

Gross pathology examination was performed in all animals.

Neuropathology evaluation: 15 days following treatment, surviving animals were sacrificed and following tissues were examined histopathologically: coronal slices of brain, cross and longitudinal slices of spinal cord, Gasserian ganglia, numerous dorsla root ganglia and associated nerve roots, sections of peripheral nerves (sciatic, peroneal, sural and tibial).

Findings

Survival: 5/15 males and 2/15 females at the high dose died on the day of treatment. In these animals no gross necropsy observations related to the treatment were noted, but microscopic examination of tissues was not performed since these tissues could not be fixed by perfusion. No other deaths occurred during the study.

Clinical signs of toxicity were only observed in the top-dose males (418 mg/kg bw) and females (209 mg/kg bw) on the day of dosing: tremors, urogenital area wetness and salivation were the main effects noted in both sexes. They did not persist after the day of dosing.

Mean body weight for top-dose males (418 mg/kg bw group) decreased by approximately 5% at the 7- and 14-day period (statistically non significantly).

FOB findings:
In the top-dose males and females (i.e. 418 mg/kg bw males, 209 mg/kg bw females) at the 3-hour post treatment evaluation following signs were observed:

- whole body tremors (both sexes)
- decreased fore limb grip strength (both sexes)
- decreased hind limb grip strength (male only)
- decreased hind leg splay (female only, not statistically significant)
- altered gait (male only, not statistically significant).

Also, effects on the autonomic nervous system were noted in this group: nasal secretion and increased rectal temperature.

In the mid-dose group females (66 mg/kg bw), fine tremors of the head or whole body were observed (in 3/15 females).

There were no treatment-related changes observed in any other treatment group or at any other measurement period.

Motor activity: Treatment related changes were limited to the 4-hour post-treatment evaluation.
In the top-dose males and females, treatment-related effects were observed: greater than 2-fold increase in total fine movement, which was presumed to be due to tremors, and decreased rearing and ambulation. Decreased rearing and ambulation were also observed in mid-dose males.

Gross pathology: No treatment-related differences were present in the absolute and relative mean brain weights and no gross treatment-related lesions were noted.

Neuropathology evaluation: Microscopic changes (details provided below) were limited to sections of the sciatic nerve and its branches (tibial and peroneal nerve), except that in one high dose female rat with peripheral nerve changes there was also evidence of vacuolation within the myelinated fibres of the caudate nucleus/putamen and within the cochlear nucleus.

The histomorphologic changes within the peripheral nerve sections indicated the presence of scattered degenerating nerve fibres or myelin sheaths. For all but the one high dose female rat, these changes were graded as "minimal" in degree (limited to a very small number of nerve fibres).

In RAR it is stated that peripheral nerve fibre degeneration (of minimal degrees) was found in 4/13 females at 209 mg/kg bw[4] (see table below). Although peripheral nerves changes were observed in treated groups of male rats, no clear dose-response pattern was observed.

As pointed out in the DAR and RAR, minimal degrees of peripheral nerve fibre degeneration are quite common in older rats, but these changes are generally not seen in young rats (like in 7-week-old rats as in the present study). Indeed, no such changes were observed in male and female control group in acute neurotoxicity study. In the DAR and RAR it was concluded that there was insufficient evidence to link the pyrethrins'

[4] Since 2 top-dose females died on the day of treatment, 13 females in this group were available for histopathological examination, and not 15, as stated in the tables in CLH Report, DAR, RAR and CAR. Also, from the available data in the CLH Report, DAR, RAR, and CAR, it is not clear how many animals had changes in peripheral nerves (e.g. did all changes in top-dose females shown in Table "Microscopic evaluation of peripheral nerves in sacrificed rats (at study termination) in Acute oral neurotoxicity study with pyrethrum extract in rats (KPIC/BRA, MGK, and SCJ, 1993)" were observed in 4 females of this group).

treatment to this minimal neuropathy, taking into account that no histopathological changes to nerve tissue were observed in the 13-week rat study, 8-week dog study, or 2-year rat study.

RAC notes that adverse effects in peripheral nerves following single exposure were also observed for certain pyrethroids, either with (e.g. beta-cyfluthrin) or without (e.g. esfenvalerate) the same type of effect in sub-chronic or long-term studies [5] . Nevertheless, a clear treatment-related increase in peripheral nerve fibre degeneration in females was observed at the highest dose level at which mortality also occurred. Therefore, this finding is not considered to be relevant for classification for STOT SE or STOT RE.

Microscopic evaluation of peripheral nerves in sacrificed rats (at study termination) in Acute oral neurotoxicity study with pyrethrum extract in rats (KPIC/BRA, MGK, and SCJ, 1993):

	MALES				**FEMALES**			
Dose (mg/kg bw/day)	**0**	**42**	**131**	**418**	**0**	**21**	**66**	**209**
No rats/group	15	15	15	10	15	15	15	13
SCIATIC NERVE								
Myelin degeneration/ sheath swelling	**0**	**0**	**0**	**2 (20)**	**0**	**0**	**0**	**1 (8)**
minimal focal	0	0	0	2	0	0	0	1
Myelin/axon degeneration	**0**	**2 (13)**	**1 (7)**	**1 (10)**	**0**	**0**	**0**	**4 (31)***
minimal focal	0	2	1	1	0	0	0	0
minimal multifocal	0	1	1	1	0	0	0	3
moderate multifocal	0	1	0	0	0	0	0	1
PERONEAL NERVE								
Myelin/axon degeneration	**0**	**0**	**0**	**0**	**0**	**1(7)**	**0**	**2 (15)**
minimal multifocal	-	-	-	-	0	1	0	1
moderate multifocal	-	-	-	-	0	0	0	1
TIBIAL NERVE								
Myelin/axon degeneration	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2 (15)**
minimal multifocal	-	-	-	-	0	0	0	1
moderate multifocal	-	-	-	-	0	0	0	1

Data are presented as number of affected animals (Incidence in percentage in brackets, vs. control 0%).
* Statistically significant from control, Fisher's exact test (chi = 5.38, P = 0.035; calculated by

[5] In acute neurotoxicity study with esfenvalerate in rats, slight to minimal axonal degeneration and/or demyelination with Schwann cell proliferation in peripheral nerves were noted at the highest dose at which mortality was also observed. However, no such changes were observed in 90-day dietary neurotoxicity study in rats or in 2-year combined chronic toxicity/carcinogenicity study in rats (RAC Opinion on esfenvalerate, 2019).
Slight axonal degeneration of single nerve fibres in the sciatic nerve was observed in 8 out of 40 animals in a 90-d rat dietary study with beta-cyfluthrin at 61/68 mg/kg bw/d (m/f). However, minimal single fibre degeneration in the sciatic nerve was also observed in 6 out of 8 rats (vs. none in controls) already after a single gavage dose of 80 mg/kg bw cyfluthrin (RAC Opinion on beta-cyfluthrin, 2020).

RAC)

Neurotoxicity observed in other studies

Clinical signs of neurotoxicity were noted during or shortly after each oral or inhalation exposure in acute and repeated-dose studies, disappeared usually shortly after cessation of exposure (e.g. latest till the next day in the acute oral toxicity studies), and were observed at dose levels below those that triggered acute oral and inhalation toxicity classification.

The proposed ATE for acute oral toxicity was 730 mg/kg bw for total pyrethrins, and lethality began to occur at around 200 – 400 mg total pyrethrins/kg bw in acute oral study in rats and in acute oral neurotoxicity study in rats.

The proposed ATE for acute inhalation toxicity was 2.6 mg/L for total pyrethrins.

Neurotoxic symptoms were noted at doses that were approximately one order of magnitude lower than the ATE for acute oral or inhalation toxicity at:

- 66 mg total pyrethrins/kg bw in females in the acute oral neurotoxicity study in rats;
- 90 – 98 mg total pyrethrins/kg bw/day in the 8-week dietary dose range finding study in dogs;
- 78 mg total pyrethrins /kg bw/day in the dose range finding study for the rat teratology study;
- 100 mg total pyrethrins/m^3/day, i.e. 0.1 mg/L/day, in the 90-day inhalation toxicity study in rats during exposure period (in inhalation chamber).

Although it is not specified at which day of the treatment neurological clinical signs started in the 8-week dietary dose range finding study in dogs, the range finding study for the rat teratology study, and the 90-day inhalation toxicity study in rats, it is stated in the RAR that neurotoxic symptoms were noted during or shortly after each oral or inhalation exposure dose.

Neurotoxic symptoms were described in humans, as well, as stated in the DAR: "massive ingestion may precipitate a neurologic syndrome ranging from numbness, excitability, tremors, and incoordination to paralysis and/or seizures". The CLH Report briefly described exposure incidents (most of which (93%) were unintended exposures), in which neurologic symptoms were among the ones most frequently reported (9.7%).

Comparison with the criteria

According to the CLP criteria, a substance is classified as STOT SE 1 if it produces 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 at generally low exposure concentrations. The hazard, according to these criteria, may not be life-threatening but could indicate functional impairment.

Guidance value ranges for STOT SE 1 are:

≤ 300 mg/kg body weight in rat, oral exposure

≤ 1.0 mg/L/4h in rat, inhalation exposure for dust/mist/fume

However, since the duration of daily exposure in 90-day inhalation toxicity study in rats was 6h instead of 4h, Haber's rule was applied (as recommended in ECHA CLP Guidance, section 3.1.2.2.), obtaining guidance values of 0.67 mg/L/6h.

Doses at which neurological symptoms (clinical signs or effects observed in FOB or motor activity) occurred are well within the guidance value range for classification, and well below the doses warranting the classification for Acute Tox. 4; H302: Harmful if swallowed, and Acute Tox. 4; H332: Harmful if inhaled.

RAC, therefore concludes that the classification for **STOT SE 1, H370 (nervous system)** is warranted. This is supported by the mode of action responsible for pyrethrins' acute toxicity described above, as well as their similarities in toxicodynamics and kinetics between humans and experimental mammals.

Respiratory tract irritation

In 90-day inhalation toxicity study in rats, dose-dependent increases in incidences and severities of squamous/squamoid metaplasia/hyperplasia in the larynx mucosa pseudostratified columnar epithelium and in the larynx ventral diverticulum cuboidal/columnar epithelium were observed, both in males and females, starting at the lowest dose tested (11 mg/m^3, i.e. 0.01 mg/L) (please see the tables in section "Additional key elements"). At this dose level there were no other treatment-related effects.

A question arises whether these changes are an adaptive response to repeated acute exposures to an irritative substance (warranting STOT SE 3 classification), or they represent specific target organ toxicity arising from repeated inhalation exposure to pyrethrins (potentially triggering STOT RE classification).

There is also a question if pyrethrins induce respiratory tract irritation in human subjects following acute exposure.

<u>Animal data on respiratory tract effects of pyrethrins</u>

Acute inhalation toxicity study in rats does not provide sufficient information on respiratory tract irritation by pyrethrins. Available study summary only reports that in all exposed groups, including the one in which no mortality was observed (0.69 mg/L total pyrethrins), laboured breathing, excessive salivation, decreased activity, and eye closure were observed immediately after exposure. Nevertheless, similar symptoms (without further specification) were also observed in vehicle (acetone) control group, while the incidences of clinical symptoms in control and exposed groups were not reported.

In **90-day inhalation toxicity study in rats**, in all exposure groups (0.01 – 0.36 mg/L) and also in the air control group, inflammation, oedema, haemorrhage, emphysema, macrophages, lymphoid cells, mineralisation, glandular dilation, and/or goblet cell hyperplasia were seen in one or more of the following tissues: nasoturbinates, nasopharynx, larynx, and the lungs. The effects were graded from minimal to moderate for animals in all study groups. However, there are no information on the incidences of described changes.

The dose-related increase in the incidence and severity of squamous/squamoid metaplasia/hyperplasia in the larynx mucosa pseudostratified columnar epithelium and in the larynx ventral diverticulum cuboidal/columnar epithelium, started at the lowest dose tested (0.01 mg/L).
Clinical signs were observed at and above 0.03 mg/L, during the in-chamber observations and during the weekly detailed observations. At lower dose levels (0.03 and 0.1 mg/L) these were secretory signs, such as nasal discharge and dried material in the facial area, in both males and females. In the top-dose rats (0.36 mg/L), in addition to secretory signs, laboured breathing, excess lacrimation, tremors, increased activity, and matted coat were also observed.

Nasal discharge and dried material in the facial area could be also signs of autonomic nervous system toxicity, and they were also noted in oral toxicity studies with pyrethrins (e.g. acute oral toxicity study in rats; acute neurotoxicity study in rats; oral neurotoxicity probe study with Pyrethrum Extract in rats). However, RAC considers that nasal secretory changes noted in the 90-day inhalation toxicity study in rats, during the in-chamber observations and in the absence of clinical signs clearly indicating neurotoxicity (e.g. excess lacrimation, tremors, increased activity), suggest respiratory irritation rather than neurotoxicity of pyrethrins. Namely, tremors seem to be more sensitive signs of pyrethrins' neurotoxicity than secretory signs, since in oral toxicity studies it was observed at doses lower than those at which nasal secretory changes were recorded (e.g. in acute neurotoxicity study in rats and in oral neurotoxicity probe study with Pyrethrum Extract in rats). The report, nevertheless, does not state whether clinical symptoms started already at the beginning of the study and for how long they lasted after exposure.

Laboured breathing was observed only at high doses in inhalation and oral toxicity studies (13-week dose range finding study in mice; rabbit teratology study; oral neurotoxicity probe study with Pyrethrum Extract in rats), concomitantly with neurological signs, so it is not considered indicative of respiratory tract irritation but of systemic toxicity of pyrethrins.

As discussed in the CLH Report and in the DAR, and as stated in ECHA CLP Guidance[6], histopathological changes observed in the larynx, nasoturbinates, and nasopharynx in 90-day inhalation toxicity study in rats could be considered as localised responses, indicative of respiratory irritation. Toxicological relevance of the upper respiratory tract changes in rodent species has been assessed in the literature (e.g. Renne et al., 2007; Osimitz et al., 2007). Renne et al. (2007) state that "rats and mice exposed via inhalation to toxic or irritating drugs, chemicals, or environmental contaminants have a relatively high incidence of lesions in the respiratory tract" and that "the most frequent target tissues include the mucosa of the nasal cavity and larynx". Larynx consists of areas that transit from a relatively durable stratified squamous epithelium to a much more fragile respiratory epithelium, and is among the one of the most sensitive sites for cellular changes in rodents inhaling xenobiotics (Renee et al., 2007). Exposure to

[6] "Category 3 effects should be confined to changes, whether functional or morphological, occurring in the upper respiratory tract (nasal passages, pharynx and larynx). Localized irritation with associated adaptive responses (e.g., inflammation, epithelial metaplasia, goblet cell hyperplasia, proliferative effects) may occur and are consistent with Category 3 responses."

irritants can induce oedema, inflammation, and, if prolonged and severe enough, ulceration, necrosis, epithelial sloughing, and even death due to occlusion of the laryngeal lumen from oedema and inflammation (Renee et al., 2007). Repeated inhalation of irritating substances could eventually lead to squamous metaplasia and hyperplasia of the transitional epithelium. Depending on severity and duration of exposure, this metaplastic epithelium may also become hyperkeratotic. Severity of squamous metaplasia/hyperplasia depends on concentration of the inhaled substance and exposure duration (Renee et al., 2007). The authors point out that although squamous metaplasia and hyperplasia of laryngeal epithelium are frequently reported in repeated-dose inhalation studies in rodents, progression to neoplasia at this site with repeated exposures is very rare. The authors conclude that "the metaplastic change by itself is simply a response to repeated irritation in which a resistant type of epithelium replaces a susceptible one".

Osimitz et al. (2007) discussed the adversity of these changes in light of using them as endpoints for quantitative risk assessment (e.g., in the US EPA assessments). After reviewing the literature, the authors concluded that the development of lesions in the upper respiratory tract in rodents was more influenced by the dose of a substance than its chemical nature; squamous metaplasia and hyperplasia in the larynx did not progress to more serious cellular changes; and the studies demonstrated partial or complete regression of laryngeal squamous metaplasia and hyperplasia following recovery period. Nevertheless, in case of pyrethrins, there is no data on reversibility of observed laryngeal changes (e.g., the study described above did not include a recovery group).

RAC, however, points out that the European Society of Toxicologic Pathologists (ESTP) at the International Expert Workshop on Squamous Metaplasia in the Rodent Larynx in 2006, concluded that slight cases of a non-diffuse laryngeal squamous metaplasia could be regarded as adaptive and non-adverse, as laryngeal dysfunction is not to be expected. On the other hand, cases of "diffuse moderate to severe squamous metaplasia should be considered adverse as it may be associated with dysfunction" (Kaufmann et al., 2009). In 90-day inhalation toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1992), moderate to moderately severe squamous/squamoid metaplasia/hyperplasia was observed in the larynx mucosa pseudostratified columnar epithelium already at the doses well below the ones related to systemic toxicity. The increase in the incidence of these laryngeal changes was dose dependent. RAC, therefore, concludes that observed laryngeal changes should be considered as adverse effects related to repeated dosing of pyrethrins, and not as an adaptive response to a respiratory irritant.

<u>Human data on respiratory tract effects of pyrethrins</u>

Almost all products with pyrethrins as an active substance also contain a synergist, principally piperonyl butoxide, which has a harmonised classification for respiratory tract irritation (STOT SE 3, H335). This fact severely complicates the assessment of potential respiratory tract irritation effect of pyrethrins in human population.

However, the US EPA report (2004) used the data from the Poison Control Centers, covering the period 1993 – 1998, which were coded in a way that pyrethrin products with or without piperonyl butoxide could be examined separately. The table below presents the number and percentage of poisoning/exposure cases with respiratory tract

symptoms out of pyrethrin exposure cases for which medical outcome (symptoms / effects occurred) was known,. The authors took into consideration only the cases with moderate or major symptoms or fatal cases. Both occupationally and non-occupationally exposed populations are included, with age ranges from children below the age of six to adults. Only the cases exposed to pyrethrins alone (without co-exposure to piperonyl butoxide) are presented in the table. The authors excluded the cases with exposure to multiple products, as well as cases with medical outcomes unrelated to pyrethrin exposure.

Number and percentage of poisoning/exposure cases with respiratory tract symptoms out of pyrethrin exposure cases for which medical outcome was known in the US EPA report from the Poison Control centers: **Symptom**	**Number of cases with a symptom**	**Percentage out of moderate/major/ fatal cases** (N = 760)	**Percentage out of cases with known medical outcome** (N = 7175)
Dyspnoea	111	15%	1.5%
Cough/choke	83	11%	1.2%
Throat irritation	49	6%	0.7%
Bronchospasm	39	5%	0.5%
Chest pain	23	3%	0.3%

Only the symptoms for moderate, major or fatal cases are presented.

There are, nevertheless, numerous limitations related to this data, including:

- It is not possible to calculate the true incidences based on poison control center data (contacting a center is not obligatory; it is done only in the case when a patient or medical professional requires expert advice);
- data regarding circumstances of exposure, symptoms, patient's underlying health status and risk factors, medical treatment, and the outcome could be limited;
- some symptoms could be related to respiratory sensitisation, rather than respiratory tract irritation (e.g. bronchospasm)[7].

Conclusion

Evidence for respiratory tract irritation from available human and animal data is too weak to conclude on the respiratory tract irritation properties of pyrethrins. Incidences of respiratory tract symptoms in the human population are rather low, and could not be clearly delineated from respiratory hypersensitivity reactions. Although animal data provide some evidence of respiratory tract irritation, they are also rather limited. Namely, the day of onset of nasal discharge observed in the 90-day inhalation study in rats is not reported, and histopathological changes were assessed only at the end of the exposure period. Therefore, RAC concludes that no classification for STOT SE 3 for

[7] There are some indications that pyrethrum extract could induce respiratory hypersensitivity reactions in human subjects. In the DAR, it is stated that an asthma-like reaction and allergic rhinitis occurred in sensitised patients, and that pyrethrum may cause hypersensitivity pneumonitis (HSDB database (2001). Also, there are case reports describing fatal outcome due to asthma attacks following exposure to pyrethrins (Wax and Hoffman, 1994; Wagner, 2000).

respiratory tract irritation is warranted.

Narcotic effects

Classification for narcotic effects is warranted for transient non-lethal effects caused by central nervous system depression after a single dose and these are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2. In acute neurotoxicity study reduced rearing and ambulation was observed in high-dose males and females at lethal doses and at lower doses they were associated with other acute neurotoxic effects warranting STOT SE 1 for nervous system. Furthermore, none of the reported effects in other toxicity studies (including oral neurotoxicity probe study (1993) and comparative FOB study of 12 commercial pyrethroid insecticides (2009); briefly described in "Supplemental information - In depth analyses by RAC", see background document) are considered to meet the CLP criteria for STOT SE 3; H336. RAC, therefore, agrees with the Dossier Submitter that there are no effects warranting the classification of Chrysanthemum cinerariaefolium extract as STOT SE 3 for narcotic effects.

Two studies described in the CLH report, provided only some supportive evidence for neurotoxicity of pyrethrins due to their limitations (e.g., non-guideline studies, low number of animals per group, unknown batch and purity of the test substance).

Comparison with the criteria

Since there is no indication that *Chrysanthemum cinerariaefolium* extract induces narcotic effects in the absence of more severe neurotoxic effects warranting STOT SE 1, and since human and animal data are too limited to indicate respiratory tract irritation, but rather suggest adverse respiratory changes following repeated exposure (addressed under STOT RE), RAC concludes that **no classification is warranted for STOT SE 3** for *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent.

Supplemental information - In depth analyses by RAC

The following two studies were also described in the CLH report, but due to their limitations (e.g., non-guideline studies, low number of animals per group, unknown batch and purity of the test substance), they can only serve as supportive evidence for neurotoxicity of pyrethrins.

Oral neurotoxicity probe study with Pyrethrum Extract in Rats (1993)

This is a GLP-compliant non-guideline study, which was performed with Pyrethrum Extract (FEK-99 blend; purity: 57.467%) in two phases. In Phase I, 1 male and 1 female CD rat/dose, were dosed by oral gavage, over a broad range of doses to generate preliminary information which doses to select for more extensive testing in Phase II. In Phase I, clinical signs of toxicity were monitored hourly for several hours after dosing. In Phase II, groups of 2-4 rats/sex/group were employed and clinical signs of toxicity were recorded and specific evaluations for tremors, arousal state, and gait were made at least hourly for several hours and 24 hours after dosing. In Phase I, males were exposed from

209 to 2610 mg/kg bw total pyrethrins (200 to 2500 mg/kg bw as the sum of pyrethrins I and II) and females from 52 to 835 mg/kg bw total pyrethrins (50 to 800 mg/kg bw as the sum of pyrethrins I and II). In Phase II, males received up to 418 mg/kg bw total pyrethrins (400 mg/kg bw as the sum of pyrethrins I and II) and females up to 209 mg/kg bw total pyrethrins (200 mg/kg bw as the sum of pyrethrins I and II).

Findings: In Phase I, mortality occurred at 1462 and 2610 mg/kg bw total pyrethrins in males, and at ≥209 mg/kg bw total pyrethrins in females. No mortality was observed in Phase II of the study.
Tremors were observed in all males and females in Phase I and in high-dose animals in Phase II. They started within few hours after the treatment and disappeared within one day after the treatment. Other clinical signs and neurotoxicity changes included gait alteration (described as "walks on toes"), piloerection, red extremities, perinasal encrustation, and salivation.

Comparative functional observational battery study of twelve commercial pyrethroid insecticides in male rats following acute oral exposure (1992)

In a GLP-compliant non-guideline study, 12 commercial pyrethroid insecticides, both type I and type II, as well as pyrethrum extract (unknown batch and purity), were evaluated for acute neurobehavioral toxicity, to determine whether they act by a common mechanism of toxicity.
Male Sprague–Dawley rats (10 per dose group) were administered a single dose of pyrethroid by oral gavage (in corn oil), at two to three dose levels, from minimally toxic to a more toxic dose.

Findings: Based on a review of the FOB data, analysed by Principal Component Analysis (PCA), and other published data, two common mechanism groups are proposed: Group 1 (T (fine tremors) syndrome; without α-cyano substituent in the 3-phenoxybenzyl alcohol moiety of the molecule), which includes pyrethrins, bifenthrin, resmethrin, permethrin, S-bioallethrin and tefluthrin, and Group 2 (CS (choreoathetosis and salivation) syndrome; with an α-cyano substituent), which includes cypermethrin, deltamethrin, esfenvalerate, b-cyfluthrin and l-cyhalothrin. Fenpropathrin exhibited features of both groups.
Study authors concluded that the potency of the α-cyano-containing pyrethroids was generally higher than the non-cyano pyrethroids, with pyrethrins as the least toxic among all tested substances.

A2.2.5.4 Overall conclusion on acute toxicity related to risk assessment

Value used in the Risk Assessment – Acute systemic toxicity	
Value	Rat LD_{50} oral = 700 mg/kg bw total pyrethrins (1073 mg/kg bw extract) Rat LD_{50} dermal = > 2000 mg/kg bw total pyrethrins (3064 mg/kg bw extract) Rat LC_{50} inhalation = 2.5 mg/L total pyrethrins (3.8 mg/L extract)
Justification for the selected	Lowest LD_{50} or LC_{50} values.

value	
Proposed classification	Acute Tox. 4, H302: Harmful if swallowed Acute Tox. 4, H332: Harmful if inhaled.

Value/conclusion used in the Risk Assessment – Acute local effects	
Value/conclusion	Not applicable
Justification for the selected value/conclusion	-

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

Based on the results of two reliable acute oral toxicity studies in rats (Kenya Pyrethrum Information Company (KPIC) study; Botanical Resources Australia Pty Ltd. (BRA), McLaughlin Gormley King Company (MGK) and SC Johnson & Son Inc. (SCJ) study), the Dossier Submitter concluded that *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent exhibited low acute oral toxicity in male rats and moderate acute oral toxicity in female rats. Transient clinical signs of toxicity in surviving animals were hyperactivity, ruffled fur and tremors.

According to the CLP Annex I 3.1.2.1. (Table 3.1.1), classification in acute oral toxicity category 4 is warranted within the range of 300 mg/kg bw < ATE ≤ 2000 mg/kg bw. Based on the lowest LD_{50} value observed (700 mg/kg bw for the sum of pyrethrins I and II in females in KPIC study) as the proposed acute toxicity estimate (ATE), the Dossier Submitter proposed to classify *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent as Acute Tox. 4, H302: Harmful if swallowed.

Acute dermal toxicity

One acute dermal toxicity study was described in the CLH Report (KPIC/BRA, MGK and SCJ). The study (limit test) was considered reliable by the Dossier Submitter (reliability 1). There was no lethality, and no systemic effects were observed. Slight to well-defined erythema, slight oedema, and stained test site were observed, but these changes withdrew after several days.

According to the CLP Annex I 3.1.2.1. (Table 3.1.1) no classification for acute dermal toxicity is required for substances with an ATE > 2000 mg/kg bw. The Dossier Submitter concluded that since the LD_{50} values for both sexes of rabbits were >2000 mg/kg bw for the sum of pyrethrins I and II, the classification for acute dermal toxicity was not warranted.

Acute inhalation toxicity

One acute inhalation toxicity study is described in the CLH Report (KPIC/BRA, MGK and

SCJ). The study was considered reliable by the Dossier Submitter (reliability 1). Inhalation exposure to different concentrations of *Chrysanthemum cinerariaefolium* extract as an aerosol to rats resulted in LC_{50} in males of 3.9 mg/L (95% confidence interval: 2.1-7.2 mg/L) for the sum of pyrethrins I and II, and in LC_{50} in females of 2.5 mg/L (1.5-4.3 mg/L) for the sum of pyrethrins I and II.

According to the CLP Annex I 3.1.2.1. (Table 3.1.1), classification in acute inhalation toxicity category 4 for dusts and mists is warranted within the range of 1.0 mg/L < ATE ≤ 5.0 mg/L. Based on the lower LC_{50} value observed in females (2.5 mg/L for the sum of pyrethrins I and II) as the proposed ATE, the Dossier Submitter proposed to classify *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent as Acute Tox. 4, H332: Harmful if inhaled.

Comments received during consultation

One Member State Competent Authority (MSCA) noted that one acute oral toxicity study described in the DAR is missing in the CLH Report. This study is described in the Background Document.

Assessment and comparison with the classification criteria

Acute oral toxicity

The Dossier Submitter based their assessment of acute oral toxicity on the two studies of high reliability, KPIC (1991), and BRA, MGK and SCJ (1992). In the DAR there is a third study (Anonymous 1986) which was not considered by the Dossier Submitter, but which is briefly described in this Opinion. (Please see the Background Document for more detailed information.)

1st oral acute toxicity study (KPIC, 1991; described in the CLH Report)

In this GLP-compliant study, performed according to OECD TG 401 (reliability 1), Sprague-Dawley rats (5 per dose/sex) were dosed with *Chrysanthemum cinerariaefolium* extract (57.574% purity) at concentrations between 929 – 3700 mg/kg bw total pyrethrins (891 and 3550 mg/kg bw as the sum of pyrethrins I and II) in males, and at concentrations between 239 – 929 mg/kg bw total pyrethrins (229 and 891 mg/kg bw as the sum of pyrethrins I and II) in females, as a single gavage.

Clinical signs comprised tremors and sometimes dark nasal (and ocular) staining and ruffled skin, and they were observed at ≥1469 mg/kg bw total pyrethrins in males, and at ≥370 mg/kg bw total pyrethrins in females. One female was found dead at 370 mg/kg bw total pyrethrins. Latest at day 2 all surviving animals appeared normal again.

At necropsy, yellow liquid in the lower gastrointestinal tract (occasionally also in the stomach), and, mainly at the highest dosage groups, clear or dark nasal staining, haemorrhagic lungs, dark genital staining and gas in the lower gastrointestinal tract were observed.

The oral LD_{50} values were found to be:

- 2230 mg/kg bw (95% confidence interval: 1803 – 2751 mg/kg bw) for total pyrethrins in males [2140 mg/kg bw (1730 – 2640 mg/kg bw) for the sum of pyrethrins I and II], and;
- 730 mg/kg bw (95% confidence interval: 521 – 1032 mg/kg bw) for total pyrethrins in females [700 mg/kg bw (500 – 990 mg/kg bw) for the sum of pyrethrins I and II].

2nd oral acute toxicity study (BRA, MGK, and SCJ, 1992; described in the CLH Report)

In this GLP-compliant study, performed according to OECD TG 401 (reliability 1), Sprague-Dawley rats (5 per dose/sex) were dosed with *Chrysanthemum cinerariaefolium* extract (57.574% purity) at concentrations between 740 – 5211 mg/kg bw total pyrethrins (710 – 5000 mg/kg bw as the sum of pyrethrins I and II) in males, and at concentrations between 329 – 2084 mg/kg bw total pyrethrins (316 – 2000 mg/kg bw as the sum of pyrethrins I and II) in females, as a single gavage.

Clinical signs in males comprised ruffling and tremors and were observed at ≥929 mg/kg bw total pyrethrins (≥891 mg/kg bw as the sum of pyrethrins I and II). In females, tremors and hyperactivity were observed at ≥521 mg/kg bw total pyrethrins (≥500 mg/kg bw as the sum of pyrethrins I and II). Latest at day 2 all surviving animals appeared normal again.

Necropsy findings were the same as the ones observed in KPIC (1991).

The oral LD_{50} of Pyrethrum extract was found to be:

- 2470 mg/kg bw (95% confidence interval: 1751 – 3491 mg/kg bw) for total pyrethrins in males [2370 (95% confidence interval: 1680 – 3350) mg/kg bw for the sum of pyrethrins I and II];
- 1073 mg/kg bw (95% confidence interval: 896 – 1292 mg/kg bw) for total pyrethrins in females [1030 (95% confidence interval: 860 - 1240) mg/kg bw for the sum of pyrethrins I and II].

3rd oral acute toxicity study (1986; described in the DAR only)

This is a pre-GLP study (GLP was not compulsory at the time when the study was performed), performed according to the protocol similar to 40 CFR, Sect. 163.81-1, Fed. Reg., August 22, 1978; modified in accordance with revised EPA Pesticide. DAR's Rapporteur Member State considered the study acceptable.

Outbred Sprague-Dawley rats (5 per dose/sex) were dosed with *Chrysanthemum cinerariaefolium* extract (55.99% purity) at concentrations between 1072 – 6762 mg/kg bw total pyrethrins (1000 and 6310 mg/kg bw as the sum of pyrethrins I and II in males, and at concentrations between 676 – 2690 mg/kg bw total pyrethrins (630 – 2510 mg/kg bw as the sum of pyrethrins I and II) in females.

The main signs of intoxication were increased responsiveness to external stimuli, tremors, salivation and ruffled appearance. It was not stated at which doses clinical signs were observed. Gross pathology showed congested lungs (from 4265 mg total pyrethrins/kg bw onwards in males, and from 1350 mg total pyrethrins/kg bw onwards in females).

Oral LD_{50} in males was stated to be 4083 mg/kg bw for total pyrethrins (3810 mg/kg bw for the sum of pyrethrins I and II), and in females 1297 mg/kg bw for total pyrethrins (1210 mg/kg bw for the sum of pyrethrins I and II).

Comparison with the criteria

ECHA CLP Guidance on the Application of the CLP criteria, 2017 states that, in general, classification is based on the lowest ATE value available. The date presented above systematically indicate that female rats are more susceptible to toxic effects of *Chrysanthemum cinerariaefolium* extract compared to male rats. RAC therefore, agrees with the Dossier Submitter's proposal to classify *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent as Acute Tox. 4, H302: Harmful if swallowed, based on the ATE of 730 mg/kg bw for total pyrethrins[8] in female rats in KPIC (1991) study (as the lowest LD_{50} value observed in acute oral toxicity studies with pyrethrin extracts). This is clearly within the range of 300 mg/kg bw < ATE ≤ 2000 mg/kg bw, defined in the CLP Annex I 3.1.2.1. (Table 3.1.1) as the ATE range for acute oral toxicity category 4. RAC also notes that LD_{50} values obtained for female rats in all three available studies (both for total pyrehtrins and for the extract) are within this range.

In conclusion, RAC concludes that classification as **Acute Tox. 4, H302: Harmful if swallowed** is warranted, with an **ATE of 730 mg/kg bw for total pyrethrins.**

Acute dermal toxicity

In this GLP-compliant study, performed according to OECD TG 402 (limit test), 5 male and 5 female New Zealand White Rabbits with healthy intact skin were tested. Approximately 24 hours before testing, the hair in the backs of the test animals were clipped. The test material, Pyrethrum Extract (57.574% Pyrethrins), was administered as a single occluded dermal application (via large porous gauze patch which covered 10% of the body surface) at a dose of 2084 mg/kg bw total pyrethrins (2000 mg/kg bw as the sum of pyrethrins I and II). After a 24h contact period, the test material was removed using deionized water.

No mortalities occurred. Very slight to well-defined erythema and very slight to slight oedema were found after unwrapping at 24 hours after treatment. From day 6 onwards no clinical signs were visible any longer, and no gross abnormalities were observed at necropsy performed 14 days after treatment.

In both males and females, the dermal LD_{50} was >2084 mg/kg bw total pyrethrins (2000 mg/kg bw as the sum of pyrethrins I and II).

Comparison with the criteria

RAC agrees with the Dossier Submitter that **classification for acute dermal toxicity is not warranted**, since dermal LD_{50} was above the range for Acute Tox. 4 for dermal toxicity (1000 < ATE ≤ 2000) set by the CLP Annex I 3.1.2.1. (Table 3.1.1).

[8] **Total pyrethrins** includes pyrethrins (total pyrethrins: EC number 232-319-8, CAS number 8003-34-7), plant material, BHT and water. **Extract** is the test substance, which includes, in addition to total pyrethrins, also the solvent.

Acute inhalation toxicity

In this GLP-compliant study, performed according to OECD TG 403, Sprague-Dawley rats (5 per dose/sex) were exposed to four-hour, whole-body inhalation exposure to *Chrysanthemum cinerariaefolium* extract (57.574% purity) as a liquid aerosol, using acetone as a vehicle, at analytical concentrations of 0, 0.7, 2.2, and 4.8 mg/L total pyrethrins (0, 0.69, 2.1, and 4.6 mg/L as the sum of pyrethrins I and II), which resulted in mortalities of 0%, 0%, 20% and 70%, respectively. The average mass median aerodynamic diameter was 2.6 µm with an average geometric standard deviation of 2.2 µm, as recommended in the OECD TG 403 (2009 update).

No mortality was observed in a group of control animals (5/sex) which received a mixture of house-line air and acetone, indicating that a solvent vehicle did not significantly contribute to pyrethrins' toxicity.

Immediately after exposure, laboured breathing, excessive salivation, decreased activity and eye closure were observed in all groups, including vehicle control. Tremors were observed during the higher-level exposures. Surviving animals exposed to pyrethrins showed clinical signs for several days before generally recovering, while the animals of the vehicle control group recovered by the morning of test day 2.

The inhalation LC_{50} was found to be:

- 4.1 mg/L (95% confidence interval: 2.2 – 7.5 mg/L) for total pyrethrins in males [3.9 (95% confidence interval: 2.1 – 7.2 mg/L) for the sum of pyrethrins I and II];
- 2.6 mg/L (1.6 – 4.5 mg/L) for total pyrethrins in females [2.5 (95% confidence interval: 1.5 – 4.3 mg/L) for the sum of pyrethrins I and II].

Comparison with the criteria

RAC agrees with the Dossier Submitter's proposal to classify *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent as Acute Tox. 4, H332: Harmful if inhaled, based on the ATE of 2.6 mg/L total pyrethrins[9] in female rats (as the lowest LD_{50} value observed in acute inhalation toxicity study with pyrethrin extracts). This is clearly within the range of 1.0 mg/L < ATE ≤ 5.0 mg/L, defined in the CLP Annex I 3.1.2.1. (Table 3.1.1) as the ATE range for acute inhalation toxicity category 4 for dusts and mists.

In conclusion, RAC proposes classification as **Acute Tox. 4, H332: Harmful if inhaled**, with an **ATE of 2.6 mg/L for total pyrethrins.**

Supplemental information - In depth analyses by RAC

Acute oral toxicity

Mortality data for the 1st oral acute toxicity study (KPIC, 1991)

[9] **Total pyrethrins** include pyrethrins (total pyrethrins: EC number 232-319-8, CAS number 8003-34-7), plant material, BHT and water. **Extract** is the test substance, which includes the solvent in addition to total pyrethrins.

Dose (sum of pyrethrins I and II) (mg/kg bw)	**Males**	**Females**
229	-	0/5
355	-	1/5
560	-	1/5*
710	-	1/5
794	-	3/5
891	0/5	5/5
1410	0/5*	-
2230	2/5	-
2660	4/5	-
3550	5/5	-

*LOAEL for clinical signs; - = not tested

Mortality data for the 2nd oral acute toxicity study (BRA, MGK, and SCJ, 1992)

Dose (sum of pyrethrins I and II) (mg/kg bw)	**Males**	**Females**
316	-	0/5
500	-	0/5*
710	0/5	-
794	-	0/5
891	0/5*	-
944	-	2/5
1120	-	3/5
1260	-	5/5
2000	-	5/5
2230	2/5	-
2660	3/5	-
3550	4/5	-
5000	5/5	-

*LOAEL for clinical signs; - = not tested

Mortality data for the 3rd oral acute toxicity study (1986)

Dose (sum of pyrethrins I and II) (mg/kg bw)	**Males**	**Females**
630	-	1/5
1000	0/5	1/5
1260	-	4/5
1580	0/5	3/5
2510	1/5	4/5
3980	2/5	-
6310	5/5	-

- = not tested

Acute inhalation toxicity

Mortality data for acute inhalation toxicity study (KPIC/BRA, MGK and SCJ)

Dose (sum of pyrethrins I and II) (mg/L)		**Mortality**	
Nominal	**Analytical** (mean±SD)	**Males**	**Females**
- (vehicle control)	- (vehicle control)	0/5	0/5
0.92	0.69 ± 0.08	0/5	0/5
5.6	2.1 ± 0.4	0/5	2/5
14.0	4.6 ± 0.5	3/5	4/5

A.2.3. Skin corrosion and irritation

An acute skin irritation study was conducted using six New Zealand White rabbits. 0.5 ml of Pyrethrum extract was applied to the back of the animals then the area was wrapped for 4 hours using a semi-occlusive dressing. The wrapping was removed at the end of the four-hour skin contact period and the residual extract was removed with deionized water. The treated areas were examined for signs of erythema and edema within 30-60 minutes after patch removal. Readings were also made after 24, 48 and 72 hours. Pyrethrum extract, when applied dermally as supplied, produced an average primary skin irritation score of 0.33 (24-72 hours) ([redacted]). (BRA, MGK and SCJ)

Score (average of 6animals investigated)	**Time**	**Erythema**	**Edema**
Average score Draize scores (0 to maximum 4)	60 min	0.67	0
	24 h	0.5	0
	48 h	0.33	0
	72 h	0.17	0
Average score	24h, 48h, 72h	0.17	0
Reversibility: *		c	c
Average time for reversibility		72 h	0

* c : completely reversible
n c : not completely reversible
n : not reversible

Table A.18 Summary table of *in vitro* studies on skin corrosion/irritation

No data are available.

Table A.19 Summary table of animal studies on skin corrosion/irritation

Summary table of animal studies on skin corrosion/irritation						
Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity), Vehicle, Dose levels Duration of exposure	Results Average score for erythema/eschar and oedema (24, 48, 72 h) per animal, observations and time point of onset, reversibility, other adverse local/systemic effects, histopathological findings		Remarks (e.g. major deviations)	Reference
US EPA 81-5, OECD 404 GLP Reliability 1 Key	Rabbit New Zealand White 6 rabbits/group (sex not reported)	Undiluted Pyrethrum Extract (FEK-99; 57.03%)	Erythema: 24 h: 0.5 48 h: 0.33 72 h: 0.17 Mean: 0.33	Edema: 24 h: 0 48 h: 0 72 h: 0 Mean: 0	-	[redacted] (KPIC) IIIA6.1.4/1 [redacted] (BRA, MGK and SCJ) IIIA6.1.4/1

Table A.26 Summary table of human data on skin corrosion/irritation

No data are available.

A2.3.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Chrysanthemum cinerariaefolium extract from HCS is not irritating.

A2.3.2 Comparison with the CLP criteria

Chrysanthemum cinerariaefolium extract from HCS does not meet the EU criteria to be classified as skin irritant. According to CLP 3.2.2.1.2.1, average primary skin irritation score (0.17) < 2.3.

A2.3.3 Conclusion on classification and labelling for skin corrosion/irritation

Not classified.

A2.3.4 Overall conclusion on skin irritation and corrosivity related to risk assessment

Conclusion used in the Risk Assessment – Skin irritation and corrosivity	
Value/conclusion	Not irritating
Justification for the value/conclusion	*Chrysanthemum cinerariaefolium* extract from HCS when applied dermally as supplied, produced an average primary skin irritation score of 0.33 (24-72 hours).
Proposed classification	Not classified

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The results of one acute skin irritation study (reliability 1), conducted in New Zealand White rabbits, showed that *Chrysanthemum cinerariaefolium* extract produced an average primary skin irritation score of 0.33 (at 24-72 hours reading) (BRA, MGK and SCJ).

The Dossier Submitter concluded that the substance, therefore, does not meet the CLP criteria to be classified as skin irritant, because the average primary skin irritation score (0.33) was < 2.3 (the threshold for classification according to the CLP Regulation, section 3.2).

Comments received during consultation

No comments were received regarding this toxicological endpoint.

Assessment and comparison with the classification criteria

In a GLP compliant study, performed according to OECD TG 404, undiluted *Chrysanthemum cinerariaefolium* extract (57.6% purity) was applied for 4 hours in a

semi-occlusive way to six New Zealand White rabbits (BRA, MGK and SCJ). After the exposure period, the residual extract was removed with deionized water. The treated areas were examined for signs of erythema and oedema within 30-60 minutes after patch removal and the readings were also made after 24, 48 and 72 hours.

In 4/6 animals very slight erythema (severity score = 1) was observed, and in one of them erythema was well-defined (score = 2, at the first reading). There was no oedema and no signs of irritation were observed on day 7.

The mean scores (according to Draize) are shown in the table below. For the readings at 24 – 72h, mean scores for erythema ranged from 0 to 1.0, and were above 0 in 3/6 animals.

Individual and mean scores in the skin corrosion/irritation study (BRA, MGK and SCJ)

	Erythema and eschar formation					
Animal No	**1**	**2**	**3**	**4**	**5**	**6**
After 4h	0	0	1	2	0	1
After 24h	1	0	1	1	0	0
After 48h	1	0	1	0	0	0
After 72h	1	0	0	0	0	0
After 7 d	0	0	0	0	0	0
Mean score 24 – 72h	**1.0**	**0**	**0.7**	**0.3**	**0**	**0**

There was no oedema (the score was 0 for all animals at all readings).

RAC notes that in the DAR, another dermal irritation study in New Zealand white rabbits was described. It was an exploratory study from 1991, in which Pyrethrum extract, applied at concentrations of 25%, 50%, and 75% for 6h per day for 5 consecutive days, did not produce scores that would trigger classification for skin corrosion/irritation. However, this study is not described here in detail, since it is a non-guideline and non-GLP study with no information on test substance purity, batch number, humidity etc.

Comparison with the criteria

RAC agrees with the Dossier Submitter[10] that the results of the study with dermally applied *Chrysanthemum cinerariaefolium* extract **does not warrant classification for skin corrosion/irritation**, since the CLP Regulation criteria for this toxicological endpoint were not met (CLP Regulation, Table 3.2.2; ECHA CLP Guidance section 3.2.2.3.2.2.). Namely, skin irritation category 2 requires mean value of ≥ 2.3 - ≤ 4.0 for

[10] RAC, however, notes that in the CLH Report average scores for erythema/eschar and oedema (24, 48, 72 h) per animal were not presented, and RAC calculated these values from the data contained in the DAR.

erythema/eschar or for oedema in at least two thirds of the tested animals from gradings at 24, 48 and 72 hours after patch removal, or that inflammation that persists to the end of the observation period, normally 14 days, in at least two thirds of animals. According to the CLP Guidance, if 6 rabbits were used in the study, classification as a skin irritant (Category 2) applies if at least 4 out of 6 rabbits show a mean score per animal of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema. In the present study, however, erythema with the highest mean score of 1 was present in 3/6 animals, which was reversible by day 7, and no oedema was found.

A.2.4. Serious eye damage and Eye irritation

Eye irritation from Pyrethrum extract was investigated in a group of six New Zealand White rabbits. A volume of 0.1 ml of Pyrethrum extract was instilled into the conjunctival sac of one eye of the animals, the other untreated eye served as a control. The treated eyes were examined at 1, 24, 48, and 72 hours, and at 4 and 7 days following instillation of Pyrethrum extract. The test article produced conjunctival irritation in all rabbit eyes at 24- and 48-hour examination. No conjunctival irritation was observed in any of the test eyes by the 72-hour reading. No corneal opacity or iritis were noted during the observation period ([redacted]). (KPIC and BRA, MGK and SCJ)

	Cornea	**Iris**	**Conjunctiva**	
			redness	chemosis
Animals investigated	6	6	6	6
60 min	0	0	1	2
24 h	0	0	1	1
48 h	0	0	0.5	0.16
72 h	0	0	0	0
Average 24h, 48h, 72h	0	0	0.5	0.39
Area effected				
Maximum average score	0	0	1	2
Reversibility*	c	c	c	c
Average time for reversion	72 h	72 h	72 h	72 h

*c : completely reversible

Table A.27 Summary table of *in vitro* studies on serious eye damage and eye irritation

No data are available.

Table A.28 Summary table of animal studies on serious eye damage and eye irritation

Summary table of animal studies on serious eye damage and eye irritation								
Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity), Vehicle, Dose levels Duration of exposure	Results Average score for corneal opacity, iritis, conjunctival redness and conjunctival oedema (24, 48, 72 h) per animal, observations and time point of onset, reversibility				Remarks (e.g. major deviations)	Reference
US EPA 81-4, OECD 405 GLP Reliability 1 Key	Rabbit New Zealand White 6 rabbits/ group (sex not reported)	Undiluted Pyrethrum Extract (FEK-99; 57.03%) 7 d	Cornea 24 h: 0 48 h: 0 72 h: 0 Mean: 0	Iris 24 h: 0 48 h: 0 72 h: 0 Mean: 0	Redness Conjunctiva 24 h: 1 48 h: 0.5 72 h: 0 Mean:0.5	Chemosis 24 h: 1 48 h: 0.2 72 h: 0 Mean: 0.4	-	[redacted] (KPIC) IIIA6.1.4/02 [redacted] (BRA, MGK and SCJ) IIIA6.1.4/2

Table A.29 Summary table of human data on serious eye damage and eye irritation

No data are available.

A2.4.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The test article produced conjunctival irritation in all rabbit eyes. No conjunctival irritation was observed in any of the test eyes by the 72-hour reading. No corneal opacity or iritis was notes during the observation period.

A2.4.2 Comparison with the CLP criteria

It does not meet the EU criteria to be classified as eye irritant. According to CLP 3.3.2.1.2. (Table 3.3.2), mean values were: corneal opacity (0) and iritis (0) < 1 and redness conjunctiva (0.5) and chemosis (0.39) < 2.

A2.4.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Not classified.

A2.4.4 Overall conclusion on eye irritation and corrosivity related to risk assessment

Conclusion used in Risk Assessment – Eye irritation and corrosivity	
Value/conclusion	Not classified
Justification for the value/conclusion	No irritation noted.
Proposed classification	Not classified

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The results of one reliable (reliability 1) eye irritation study, conducted in New Zealand White rabbits, showed that *Chrysanthemum cinerariaefolium* extract produced conjunctival irritation in the eyes of all rabbits examined at 24- and 48-hours after exposure to the substance (KPIC/BRA, MGK and SCJ). No conjunctival irritation was observed in any of the test eyes by the 72-hour reading. No corneal opacity or iritis were noted during the observation period.

The Dossier Submitter concluded that the substance does not meet the CLP criteria to be classified as eye irritant, because in comparison with the criteria in the CLP Regulation, section 3.3 (Table 3.3.2), mean values for corneal opacity (0) and iritis (0) were < 1, and conjunctival redness (0.5) and chemosis (0.4) were < 2.

Comments received during consultation

No comments were received regarding this toxicological endpoint.

Assessment and comparison with the classification criteria

In this GLP study, performed in six New Zealand White rabbits according to OECD TG 405, 0.1 mL of undiluted *Chrysanthemum cinerariaefolium* extract (57.0% purity) was instilled into the conjunctival sac of one eye of the animals, while the other untreated eye served as a control (KPIC/BRA, MGK and SCJ). The eyes were not washed subsequent to the treatment. The treated eyes were examined at 1, 24, 48, and 72 hours, and at 4 and 7 days following instillation of the test substance.

Chrysanthemum cinerariaefolium extract produced conjunctival irritation in the eyes of all rabbits at the 24- and 48-hour examination. Slight conjunctival redness (grade 1) and obvious chemosis (grade 2) and discharge (grade 2-3) were observed 1h after instillation. The symptoms became milder within 24h and disappeared in the majority of animals within 48h. Within 72h all animals were free from irritation. The cornea and iris were not affected at all.

The mean scores (according to Draize) are shown in the table below. For the readings at 24 – 72h, the mean scores for conjunctival redness and chemosis ranged from 0.3 to 0.7 and were fully reversible already at study day 4.

Individual and mean scores in the eye irritation study (KPIC/BRA, MGK and SCJ)

	Conjunctiva - redness						**Conjunctiva - chemosis**					
Animal No	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
1 hour	1	1	1	1	1	1	2	2	2	2	2	2
24 hours	1	1	1	1	1	1	1	1	1	1	1	1
48 hours	0	1	0	1	1	0	0	0	0	1	0	0
72 hours	0	0	0	0	0	0	0	0	0	0	0	0
Day 4	0	0	0	0	0	0	0	0	0	0	0	0
Day 7	0	0	0	0	0	0	0	0	0	0	0	0
Mean score 24 – 72h	**0.3**	**0.7**	**0.3**	**0.7**	**0.7**	**0.3**	**0.3**	**0.3**	**0.3**	**0.7**	**0.3**	**0.3**

There were no changes in cornea or iris (the score was 0 for all animals at all readings)

Comparison with the criteria

RAC agrees with the Dossier Submitter[11] that the results of the eye irritation study with *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent **does not warrant classification for serious eye damage/eye irritation**, since the CLP Regulation criteria for this toxicological endpoint were not met (CLP Regulation, Table 3.3.2; ECHA CLP Guidance section 3.3.2.3.2.2.). Namely, eye irritation (Category 2) requires a positive response of corneal opacity ≥ 1 and/or iritis ≥ 1, and/or conjunctival

[11] RAC, however, notes that in the CLH Report average scores for eye changes (24, 48, 72 h) per animal were not presented, and RAC calculated these values from the data contained in the DAR.

redness ≥ 2 and/or conjunctival oedema (chemosis) ≥ 2 in at least two thirds of the tested animals, 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.

According to the CLP Guidance, if 6 rabbits were used in the study, classification for eye irritation (Category 2) applies if at least 4 out of 6 rabbits show a mean score per animal of ≥ 1 for corneal opacity and/or ≥ 1 for iritis and/or ≥ 2 conjunctival erythema (redness) and/or ≥ 2 conjunctival oedema (swelling) (chemosis), and these changes fully reverse within an observation period of normally 21 days.

In the present study, the highest mean score for conjunctival redness and chemosis per animal was 0.7, and the changes were fully reversible (score 0) already at study day 4.

A.2.5. Skin sensitisation

Table A.30 Summary table of studies on skin sensitisation

<table>
<tr><th colspan="6">Summary table of in vitro studies on skin sensitisation</th></tr>
<tr><td>Method, Guideline, GLP status, Reliability, Key/supportive study</td><td>Test substance (including purity), Vehicle,
Doses</td><td>Relevant information about the study (e.g. organism (e.g. bacteria), cell type, strains)</td><td>Results</td><td>Remarks (e.g. major deviations)</td><td>Reference</td></tr>
<tr><td>Similar to OECD TG 442D In vitro sensitisation assay
GLP
Reliability 1
Supportive</td><td>Pyrethrum Extract Pale (50% w/w), Lot# 2016-5-BB
50%, 25%, 10%, 5%, 2%, 1%, 0.4% and 0.2% v/v total pyrehtrins in EtOH (25%, 12.5%, 5%, 2.5%, 1%, 0.5%, 0.2%, and 0.1%)</td><td>EpiDerm™ from MatTek (reconstructed human epidermis (RHE) of normal, human-derived epidermal keratinocytes (NHEK))</td><td>SI > 2
Sensitiser
<table><tr><th>Conc.</th><th>SI</th></tr><tr><td>0.2%</td><td>0.8</td></tr><tr><td>0.4%</td><td>2.2</td></tr><tr><td>1%</td><td>1.9</td></tr><tr><td>2%</td><td>2.8</td></tr><tr><td>5%</td><td>2.8</td></tr><tr><td>10%</td><td>2.8</td></tr><tr><td>25%</td><td>4.1</td></tr><tr><td>50%</td><td>4.4</td></tr></table></td><td></td><td>Troese M., 2017 (KPIC)</td></tr>
<tr><td>Similar to OECD TG 442D In vitro sensitisation assay
GLP
Reliability 1
Supportive</td><td>Refined Pyrethrum Concentrate (53.72% w/w), Lot# 10209
50%, 25%, 10%, 5%, 2%, 1%, 0.4% and 0.2% v/v total pyrethrins in EtOH (25%, 12.5%, 5%, 2.5%, 1%, 0.5%, 0.2%, and</td><td>EpiDerm™ from MatTek (reconstructed human epidermis (RHE) of normal, human-derived epidermal keratinocytes (NHEK))</td><td>SI > 2
Sensitiser
<table><tr><th>Conc.</th><th>SI</th></tr><tr><td>0.2%</td><td>1.0</td></tr><tr><td>0.4%</td><td>1.6</td></tr><tr><td>1%</td><td>1.6</td></tr><tr><td>2%</td><td>1.7</td></tr><tr><td>5%</td><td>2.9</td></tr><tr><td>10%</td><td>7.3</td></tr><tr><td>25%</td><td>21.2</td></tr><tr><td>50%</td><td>25.3</td></tr></table></td><td></td><td>Troese M., 2017 (MGK)</td></tr>
</table>

	0.1%)				
Similar to OECD TG 442D *In vitro* sensitisation assay GLP Reliability 1 Supportive	PY-T-50 Pale Refined Pyrethrins (Pyrethrin Extract (50%, Lot# 0116-501-6101 25%, 12.5%, 5%, 2.5%, 1%, 0.5%, 0.2% and 0.1% v/v total pyrethrins in EtOH	EpiDerm™ from MatTek (reconstructed human epidermis (RHE) of normal, human-derived epidermal keratinocytes (NHEK))	SI > 2 Sensitiser Conc. / SI 0.1% / 1.2 0.2% / 1.8 0.5% / 3.0 1% / 3.3 2.5% / 4.9 5% / 6.3 12.5% / 17.2 25% / 32.2		Troese M., 2017 (BRA)
Summary table of animal studies on skin sensitisation					
Method, Duration of study, Route of exposure (e.g. topical/intradermal, induction/challenge if relevant), Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results (e.g. EC3-value or amount of sensitised animals at induction dose)	Remarks (e.g. major deviations)	Reference
US EPA 81-6 (1984), OECD 406 (1981), Modified Buehler method GLP Reliability 2 Key	Guinea pig, Hartley, Male Test substance: 10 Naive control: 10 Positive control: 10	Pyrethrum Extract Pale (FEK-99, Purity 57.03%)	0/10 Not sensitising	A minimal of 20 animals have to be used in the treatment group.	[redacted] (KPIC) IIIA6.1.5 [redacted] (BRA, MGK and SCJ) IIIA6.1.5

LLNA Method NIH No. 99-4494, 1999, EPA OPPTS 870.2600, Final Guideline (March 2003), OECD No. 429, revised July 2010. GLP Reliability 1 Key	Mice, CBA/J, female 5	Pyrethrum Extract Pale (50% w/w), Lot# 2016-5-BB Two positive control substances: α-hexylcinnamaldehyde in DMF (25% HCA) and 1-chloro-2,4-dinitrobenzene in DMF (0.25% DNCB)	EC3 = 4.0% Sensitiser	-	[redacted] (KPIC)
LLNA Method NIH No. 99-4494, 1999, EPA OPPTS 870.2600, Final Guideline (March 2003), OECD No. 429, revised July 2010. GLP Reliability 1 Key	Mice, CBA/J, female 5	Refined Pyrethrum Concentrate (53.72% w/w), Lot# 10209 Two positive control substances: α-hexylcinnamaldehyde in DMF (25% HCA) and 1-chloro-2,4-dinitrobenzene in DMF (0.25% DNCB)	EC3 = 7.1% Sensitiser	-	[redacted] (MGK)
LLNA Method NIH No. 99-4494, 1999, EPA OPPTS 870.2600, Final Guideline (March	Mice, CBA/J, female 5	PY-T-50 Pale Refined Pyrethrins (Pyrethrin	EC3 = 6.2% Sensitiser	-	[redacted] (BRA)

2003), OECD No. 429, revised July 2010. GLP Reliability 1 Key		Extract (50%)), Lot# 0116-501-6101 Two positive control substances: α-hexylcinnamaldehyde in DMF (25% HCA) and 1-chloro-2,4-dinitrobenzene in DMF (0.25% DNCB)			

Table A.31 Summary table of human data on skin sensitisation
No data are available.

Table A.20 Summary table of other studies relevant for skin sensitisation
No data are available.

A2.5.1 Short summary and overall relevance of the provided information on skin sensitisation

To assess the contact dermal sensitization potential of Pyrethrum extract, a guinea pig dermal sensitisation study (modified Buehler method) was developed. In preliminary dose-range-finding studies, 10 animals were exposed to 4 different concentrations of Pyrethrum extract at the highest non-irritating concentrations (as supplied (50%), and at 75% v/v (32.5%), 50% v/v (25%), and 25% v/v (12.5%) in corn oil). Based upon the results of the dose-range-finding studies, Pyrethrum extract was dosed as supplied for induction and challenge studies.

In the main study a group of 10 albino guinea pigs were clipped on the left side and a gauze patch loaded with Pyrethrum extract was applied and an elastic bandage was wrapped around the trunk of the animal. After 6 hours, the patch was removed, and the site was cleaned with deionised water. Examinations were performed 60 to 75 minutes and at 24 hours after patch removal using the Draize method. Animals were rested for one day before a second induction application was applied to the same skin site. This procedure was repeated three times weekly for a total of nine applications. Then animals had a two-week rest period. At the end of this rest period, a challenge application was made during 6 hours at a challenge site (on the right side). A group of 10 naïve control were treated with Pyrethrum extract in the same manner. This group served as the control challenge group. Examinations were performed 24 hours and 48 hours. Some signs of erythema were noted in various animals at different time points after each induction phase. However, no such signs were apparent at the challenge phase. The positive control, 1-chloro-2,4-dinitrobenzene, appears to be a dermal sensitizer to guinea pigs. In conclusion, Pyrethrum extract does not appear to be a dermal sensitizer to guinea pigs (Troese, 2017). (KPIC and BRA, MGK and SCJ)

Further studies

Test items were tested in an *in vitro* sensitisation assay using in ethanol (EtOH) as vehicle. A RHE of NHEK was tested for the skin sensitising properties. Due to this test focuses in the activation of keratinocytes is similar to the OECD TG 442D. However, molecular markers are different between OECD assay and applicants' assay: while OECD TG 442D measures the activation of Nrf2-ARE signalling pathway, the test performed with the RHE of NHEK measures IL-18 secretion. This cytokine is related with activation of keratinocytes in inflammatory responses and can be used to discriminate contact sensitizers from irritants and low molecular weight respiratory allergens. The positive control 1-chloro-2,4-dinitrobenzene (0.15% DNCB) induced the expected stimulation indices confirming the validity of the assay. Also, the negative (2% lactic acid), vehicle and undosed controls do not induce an increment in the stimulation index. Following 24-hour exposure to the test materials, the assay media was sampled for IL-18 analysis by ELISA kit. To calculate the IL-18 measurement (pg/ml) for each sample, the optical density of the stopped reaction was measured at a wavelength of 450 nm, subtracting out a background reading for all samples at 620 nm. A Stimulation Index (SI), i.e., the fold change in IL-18 of a test substance as compared to the vehicle control, was calculated. A substance with an SI less than 1.6 was considered a non-sensitizer; a substance with an SI greater than or equal to 2.0 was considered a sensitizer.

The viability of the tissues at 24 hours was determined using methyl thiazole tetrazolium (MTT) uptake and reduction. The absorbance of each sample was measured at 540 nm using a reference wavelength of 690 nm. The viability was then expressed as a percent of control values, corrected for direct MTT reduction.

Also, test items were tested *in vivo* in a dermal sensitisation study following OECD TG 429 using in *N,N*-dimethylformamide as vehicle. Five female CBA/J mice per dose level was tested for the skin sensitizing properties by a local lymph node assay. The positive

controls α-hexylcinnamaldehyde (HCA, 85%) and 1-chloro-2,4-dinitrobenzene (DNCB) induced the expected stimulation indices confirming the validity of the assay. The mice were given an intraperitoneal injection of the thymidine analog 5-bromo-2'-deoxy-uridine (BrdU) approximately five hours prior to euthanasia. The auricular lymph nodes were combined for each animal and single-cell suspensions were generated in RPMI-10 medium. An aliquot of each cell suspension was taken for immunophenotyping analysis; the remaining cell suspensions were fixed with 85% ethanol. The cell suspensions were used to determine BrdU incorporation into the lymphocyte and the total number of cells in the nodes, for each individual animal.

The percentages of B^+ and T^+ cells and of I-$A^{\kappa+}$ and I-$A^{\kappa+}CD69^+$ cells were determined by flow cytometry and the B:T ratios were calculated. Test groups that show an increase greater than 25% (1.25-fold) when compared to the vehicle control in these LNC surface markers and have an SI value of 3 or more are considered dermal sensitisers. The positive controls (0.25% DNCB and 25% HCA) were both found to be sensitizing.

There were no effects on body weights, clinical signs of toxicity or mortality. None of the test item treatments resulted in increases in ear thickness of 25% or more; therefore, the test items were not considered irritating. (KPIC and BRA, MGK and SCJ)

Pyrethrum Extract Pale (50% w/w) (KPIC)

Application of the test item at 0.4% (v/v) in EtOH (0.2%) onward resulted in SI values greater than 2, with the only exception of the 1.0% (v/v) (0.5%) (SI = 1.9 – probable sensitiser), existing a dose-response relationship.

Topical application of the above test item at 5% and 25% (v/v) (2.5% and 12.5%) in DMF resulted in SI values greater than or equal to 3. At a concentration of 10% (v/v) (5%) an SI of 2.9 was achieved. Treatment with the test item induced an increase of greater than 25% (1.25-fold) over the vehicle control in % B, B:T ratio, and % I-$A^{\kappa+}CD69^+$. Treatment with the test item at 25% (v/v) (12.5%) also induced an increase greater than 25% over the vehicle control in % I-$A^{\kappa+}$. The test item was only of borderline activity. However, this test item is considered a sensitizing substance. The EC3 calculated for the test item is 4.0% (v/v) (2%).

Refined Pyrethrum Concentrate (53.72% w/w) (MGK)

Application of the test item at 5.0% (v/v) in EtOH (2.5%) onward resulted in SI values greater than 2, existing a dose-response relationship. Between 0.4% and 2.0% (v/v) (0.2% and 1%) concentrations a SI greater than 1.6 (probable sensitiser) was achieved.

Topical application of the above test item at 5%, 10% and 25% (v/v) in DMF (2.5%, 5%, 12.5%) resulted in SI values greater than 3 at 10% and 25% (5% and 12.5%). Treatment with the test item at 10% and 25% (v/v) (5% and 12.5%) induced an increase of greater than 25% (1.25-fold) over the vehicle control in % B, B:T ratio, and % I-$A^{\kappa+}CD69^+$. Treatment with the test item at 25% (v/v) (12.5%) also induced an increase greater than 25% over the vehicle control in % I-$A^{\kappa+}$. Therefore, this test item is considered a sensitizing substance. The calculated EC3 of Refined Pyrethrum Concentrate (53.72% w/w) is 7.1% (v/v) (3.5%).

PY-T-50 Pale Refined Pyrethrins (BRA)

Application of the test item at 1.0% (v/v) in EtOH (0.5%) onward resulted in SI values greater than 2, existing a dose-response relationship. At 0.4% (v/v) (0.2%) a SI = 1.8 (probable sensitiser) was achieved.

Topical application of the above test item at 10% and 25% (v/v) (5% and 12.5% total pyrethrins respectively) in DMF resulted in SI values greater than 3. Treatment with the test item induced an increase of greater than 25% at 2.5% (v/v) (1.25% total pyrethrins) (in % B, B:T ratio, and % I-$A^{\kappa+}CD69^+$), 5% (v/v) (2.5% total pyrethrins) (in

B:T ratio and% I-$A^{k+}CD69^{+}$), and at 10% and 25% (v/v) (5% and 12.5% total pyrethrins respectively) (%B, B:T ratio, % I-A^{k+}, and % I-$A^{k+}CD69^{+}$). Therefore, this test item is considered a sensitizing substance. The calculated EC3 of PY-T-50 Pale Refined Pyrethrins is 6.2% (v/v) (3.1% total pyrehtrins). The SIs at 25% (v/v) (12.5% total pyrethrins) of PY-T-50 Pale Refined Pyrethrins and HCA (weak sensitiser) are 4.3 and 5.9, respectively. HCA is a typical Cat. 1B sensitiser as shown in the ANNEX 1 of OECD TG 429 *'performance standards'* where EC3 of HCA ranges from 4.4% to 14.7%. Since SI of PY-T-50 Pale Refined Pyrethrins is similar to that of HCA, both compounds are considered to have comparable potency for skin sensitisation. PY-T-50 Pale Refined Pyrethrins also should be categorized as Cat. 1B.

Mean stimulating indices observed in LLNA with Pyrethrum extracts

Test concentration	**5% (v/v)** **2.5%**	**10% (v/v)** **5%**	**25% (v/v)** **12.5%**
Pyrethrum Extract Pale (50% w/w)	3.5	2.9	3.0
Refined Pyrethrum Concentrate (53.72% w/w)	2.3	4.0	8.1
PY-T-50 Pale Refined Pyrethrins	1.8	6.7	4.3

SI ≥ 3 indicates a sensitizing response
Vehicle Control – (DMF) SI = 1.0, Positive Control – (25% HCA) 5.9, Positive Control – (0.25% DNCB) 10.1

Under the experimental conditions of these *in vivo* studies, the test items are considered sensitizing. The calculated EC3 were 4.0% 7.1%, and 6.2% (v/v) (12.5%, 3.5%, and 4.4% total pyrethrins) for Pyrethrum-Extract 50%, Refined Pyrethrum Concentrate, and PY-T-50 Pale Refined Pyrethrins respectively. However, for Pyrethrum Extract Pale 50% there was no dose response for the SI ([redacted]). (KPIC and BRA, MGK, and SCJ)

Although the results differ between Buehler method and LLNAs, active substance will be classified as skin sensitizer for different reasons: Buehler method only measures the adverse outcome in a subjective way while LLNA measures a key event in an objective way; the Buehler test only has to test one concentration while LLNA tests three different concentrations (LLNA was performed using three sources also); finally, the positive result in the LLNAs is supported by the positive result in the three *in vitro* sensitization assays.

A2.5.2 Comparison with the CLP criteria

Classified as Skin Sens. 1B (H317: May cause an allergic skin reaction) because, according to the CLP 3.4.2.2.3.3. (Table 3.4.4.), EC3 > 2 % in the three LLNA studies.

A2.5.3 Conclusion on classification and labelling for skin sensitisation

Skin sensitisation, Category 1B, H317: May cause an allergic skin reaction.

A2.5.4 Overall conclusion on skin sensitisation related to risk assessment

Conclusion used in Risk Assessment – Skin sensitisation	
Value/conclusion	Sensitising
Justification for the value/conclusion	Refer to discussion above.
Proposed classification	Skin sensitisation, Category 1B, H317: May cause an allergic skin reaction

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Seven studies assessing the skin sensitisation properties of *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent are presented in the CLH Report: three *in vitro* studies (performed on reconstructed human epidermis of normal, human-derived epidermal keratinocytes) (KPIC 2017, MGK 2017, BRA 2017), one modified Buehler test (KPIC/BRA, MGK, SCJ), and three LLNA studies (KPIC, MGK, BRA).

Out of these, only the modified Buehler test (KPIC/BRA, MGK, SCJ) showed negative results, while the LLNA and *in vitro* studies were positive.

In the modified Buehler test, also described in the DAR, no erythema or oedema was observed in any of the animals challenged with the undiluted test substance, although the positive control (1-chloro-2,4 dinitrobenzene) showed sensitising effects.

Three batches of *Chrysanthemum cinerariaefolium* extract were tested in *in vitro* assays (KPIC 2017, MGK 2017, BRA 2017) which were similar to those described in OECD TG 442D, which were considered by the Dossier Submitter as supportive evidence. Interleukin-18 (IL-18) secretion from human-derived epidermal keratinocytes was measured by ELISA. Stimulation indices (SI) obtained in all three studies clearly indicated that the test substance had skin sensitisation properties.

Three LLNA studies, using N,N-dimethylformamide as the vehicle, measured BrdU (an analogue of thymidine) incorporation into the DNA of proliferating cells in the draining auricular lymph nodes following exposure to different batches of *Chrysanthemum cinerariaefolium* extract. EC3 values, based on the SI shown in table in the section "Supplemental information - In depth analyses by RAC" in the background document, were calculated to be 4.0% in KPIC study, 7.1% in MGK study, and 6.2% in BRA study. Regarding the percentages of B+ and T+ cells and of I-Aκ+ and I-Aκ+CD69+ cells determined by flow cytometry, and the B:T ratios, all three batches showed a positive result, i.e. an increase greater than 25% (ie 1.25-fold) when compared to the vehicle control.

The Dossier Submitter concluded that although the results differ between the Buehler method and the LLNAs, the active substance should be classified as a skin sensitiser for the following reasons:

- the Buehler method only measures the adverse outcome in a subjective way while the LLNA measures a key event in an objective way;
- in the Buehler test only one concentration of the test substance was tested while in the LLNA tests three different concentrations were applied
- the LLNA was performed using test substances from three different sources; and
- the positive results in the LLNAs are supported by the positive results in the three *in vitro* sensitisation assays.

Based on the EC3 values > 2% in the three LLNA studies, the Dossier Submitter proposes classification as Skin Sens. 1B (H317: May cause an allergic skin reaction),

according to the CLP, Annex I, 3.4.2.2.3.3 (Table 3.4.4.).

Comments received during consultation

No comments were received regarding this toxicological endpoint.

During consultation of the CLH report for the *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent, however, one MSCA questioned whether a higher concentration (undiluted) for the main LLNA study should have been chosen since it cannot be ruled out that a higher concentration could have given a higher EC3 response and thereby a higher classification (i.e. Category 1A) might have been justified. The Member State, therefore, suggest the classification as Skin Sens. Category 1, H317 (without sub-categorisation).

Assessment and comparison with the classification criteria

All the skin sensitisation studies described in the CLH Report were GLP compliant, and were performed with methodology either consistent with or similar to relevant OECD TGs. Out of these studies, one study is scored by the Dossier Submitter with reliability 2 (modified Buehler test, since only 10 instead of the recommended 20 animals were used in the study), while the others are scored with reliability 1.

Modified Buehler test (KPIC/BRA, MGK, SCJ)

This is the oldest available study (from 1991), and it is the only negative study out of 7 studies presented in the CLH Report. In the preliminary dose-range-finding studies, 10 Hartley guinea pigs were exposed to 4 different concentrations of *Chrysanthemum cinerariaefolium* extract (FEK-99 blend; purity: 57.57%) at the highest non-irritating concentrations: as supplied (50%), and at 75% v/v (32.5%), 50% v/v (25%), and 25% v/v (12.5%) in corn oil. Based upon the results of the dose-range-finding studies, the test substance was dosed as supplied (undiluted) for induction and challenge applications.

In the main study, a group of 10 guinea pigs were exposed (via gauze patch loaded with *Chrysanthemum cinerariaefolium* extract) for 6 hours, after which the application site was cleaned with deionised water. Examinations were performed 60 to 75 minutes and at 24 hours after patch removal using the Draize method. There were nine induction applications in total (three per week). After a two-week rest period, a challenge application was made during 6 hours. A group of 10 naïve controls were treated with the test substance in the same manner (the control challenge group). Examinations were performed at 24 hours and 48 hours. Some signs of erythema were noted in various animals at different time points after each induction phase. However, no such signs were apparent at the challenge phase. The positive control (1-chloro-2,4-dinitrobenzene) showed a positive response. It was concluded that *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent does not appear to be a dermal sensitiser in guinea pigs.

LLNA studies (KPIC, MGK, BRA)

Three different batches of *Chrysanthemum cinerariaefolium* extract were tested in three

LLNA studies, following OECD TG 442B, and using N,N-dimethylformamide as vehicle (Study KPIC: Pyrethrum Extract Pale 50% w/w, Lot# 2016-5-BB; Study MGK: Refined Pyrethrum Concentrate 53.72% w/w, Lot# 10209; Study BRA: PY-T-50 Pale Refined Pyrethrins 50%, Lot# 0116-501-6101). In all three studies, five female CBA/J mice per dose level were tested. Once daily for three consecutive days the test substance was applied at concentrations of 5%, 10%, or 25% v/v in N,N-dimethylformamide, with percentages of total pyrehtrins of 2.5%, 5%, or 12.5% at each dose level, respectively. Higher doses were not tested, since in the pre-test (subsequently provided data, as the reply to an EFSA comment) skin irritation (alopecia, erythema) was observed at 50% pyrethrins. At 100% test concentration, lethality was also found. Also, the Applicant considered that "since EC3 value is calculated using the results of the data points lying immediately above and below the SI value of 3, EC3 value and GHS classification based on EC3 value do not change even if tested with concentrations higher than 25%, the highest dose tested".

The positive controls (α-hexylcinnamaldehyde and 1-chloro-2,4-dinitrobenzene) induced the expected stimulation indices confirming the validity of the assay. The mice were given an intraperitoneal injection of the thymidine analogue 5-bromo-2'-deoxy-uridine (BrdU) approximately 5 hours prior to euthanasia. The auricular lymph nodes were combined for each animal and single-cell suspensions were generated in RPMI-10 medium. An aliquot of each cell suspension was taken for immunophenotyping analysis; the remaining cell suspensions were fixed with 85% ethanol. The cell suspensions were used to determine BrdU incorporation into the lymphocyte and the total number of cells in the nodes, for each individual animal.

There were no effects on body weights, clinical signs of toxicity or mortality. None of the test item treatments resulted in increases in ear thickness of 25% or more, therefore, the test substance was not considered to be irritating, according to OECD TG 442B.

RAC notes that the described protocol of three LLNA studies is more in line with OECD TG 442B than with OECD TG 429, which could, potentially, influence the interpretation of the results. In OECD TG 442B, the decision process regards a result as positive when the SI ≥1.6, although the strength of the dose-response relationship, the statistical significance and the consistency of the solvent/vehicle and PC responses may also be used when determining whether a borderline result (i.e. SI value between 1.6 and 1.9) is declared positive. In OECD TG 429, the decision process with regard to a positive response includes an SI ≥3, together with consideration on the dose-response relationship. Regarding Stimulation Indices (table in the section "Supplemental information - In depth analyses by RAC", background document), the results for the three tested batches are the following:

- KPIC study – Pyrethrum Extract Pale 50% w/w, Lot# 2016-5-BB: dose-response was not observed, and SI >3 was observed at the lowest dose only, although SI were above 1.9 at all three tested doses. For the RAC opinion, an EC3 could not be calculated for this study since a normal dose range curve was not achieved.
- MGK study – Refined Pyrethrum Concentrate 53.72% w/w, Lot# 10209: a clear dose-response curve was observed, with SI >3 at 5% and 12.5% total pyrethrins (and >1.9 at all three tested concentrations), and with an EC3 value of 7.1%.
- BRA study – PY-T-50 Pale Refined Pyrethrins 50%, Lot# 0116-501-6101: again, a

clear dose-response was not found, and for the RAC opinion an EC3 value could not be calculated. Nevertheless, SI values >3 were noted at 5% and 12.5% total pyrethrins.

RAC agrees with the Dossier Submitter that the percentages of B+ and T+ cells and of I-Aκ+ and I-Aκ+CD69+ cells and calculated B:T ratios were also indicative of skin sensitising property of the test substance (data are presented and described in the CLH Report). RAC, therefore, agrees with the Dossier Submitter that results from LLNA studies show the *Chrysanthemum cinerariaefolium* extract has skin sensitising properties. Nevertheless, since the CLP criteria for sub-categorisation refer to the LLNA using radioactive labelling (OECD TG 429) and no guidance is available for the BrdU modification (OECD TG 442B), it is not possible to decide on sub-categorisation based on these results.

In vitro studies (KPIC 2017, MGK 2017, BRA 2017)

Pyrethrum extracts of different lots and different manufacturers (Study KPIC 2017: Pyrethrum Extract Pale 50% w/w, Lot# 2016-5-BB; Study MGK 2017: Refined Pyrethrum Concentrate 53.72% w/w, Lot# 10209; Study BRA 2017: PY-T-50 Pale Refined Pyrethrins 50%, Lot# 0116-501-6101) were tested in the EpiDerm™ system from MatTek on reconstructed human epidermis (RHE) of normal, human-derived epidermal keratinocytes (NHEK) in ethanol as the vehicle. Test substance concentrations ranged from 0.2% to 50% for Lot# 2016-5-BB and Lot# 10209, and from 0.1% to 25% for Lot# 0116-501-6101. The study protocol was similar to OECD TG 442D, but instead of measuring the activation of the Nrf2-ARE signalling pathway (i.e. luciferase gene induction by a skin sensitising substance) in KPIC (2017), MGK (2017), and BRA (2017) studies, IL-18 secretion was measured[12] by ELISA. A Stimulation Index (SI; in this assay the fold change in IL-18 secretion induced by a test substance as compared to the vehicle control) was calculated: a substance with an SI < 1.6 was considered a non-sensitiser; a substance with an SI ≥ 2.0 was considered a sensitiser. The positive control (0.15% 1-chloro-2,4-dinitrobenzene) induced the expected SI confirming the validity of the assay. The negative control (2% lactic acid), vehicle and undosed controls did not show positive responses. Tissue viability at 24 hours was determined using methyl thiazole tetrazolium (MTT) uptake and reduction.

The SI ranged from 0.8 – 4.4 for Lot# 2016-5-BB, 1.0 – 25.3 for Lot# 10209, and 1.2 – 32.2 for Lot# 0116-501-6101, with a clear dose-response pattern (figure in the section "Supplemental information - In depth analyses by RAC", background document), indicating that the tested substance had skin sensitising properties.

RAC notes that the protocol of these studies is not yet validated by the OECD. However, it assesses Key event 2 (release of pro-inflammatory mediators by keratinocytes) of the Adverse Outcome Pathway (AOP) for skin sensitisation (Gibbs *et al*. 2013) and it is listed among information sources that could be used within defined approaches and IATAs for skin sensitisation (OECD 2016a). Also, this prediction model provides promising performance, with levels of sensitivity, specificity and accuracy similar to OECD validated

[12] The cytokine is related with activation of keratinocytes in inflammatory responses and can be used to discriminate contact sensitisers from irritants and low molecular weight respiratory allergens.

assays (Deng *et al*. 2011, Andres *et al.* 2020). Since it has been performed on a limited number of substances, further testing of a wider range of substances should be performed (Deng *et al.* 2011, Andres *et al.* 2020).

RAC considers, therefore, that these studies provide supportive evidence for skin sensitising properties of *Chrysanthemum cinerariaefolium* extract.

RAC notes that there are human data describing skin reactions to pyrethrin extracts, which are probably of allergic origin (e.g. Mitchell *et al*. 1972, Garcia-Bravo *et al*. 1995). Nevertheless, as pointed out in a review article by Osimitz *et al.* (2009), although extensive patch testing has been done with pyrethrum extracts, most studies were performed before the current refined commercial material was available (i.e. before 1967). Therefore, it is possible that in older studies irritating levels of pyrethrins were used, possibly containing unknown impurities, leading to false-positive reactions. Additionally, only a few studies report results of testing in individuals clinically suspected of having pyrethrum allergic contact dermatitis (Osimitz *et al*. 2009). RAC concludes that the frequency of skin sensitisation to pyrethrins in the human population cannot be assessed based on presently available data, and therefore human data are not useful for the evaluation of the skin sensitising potency of pyrethrins.

Comparison with the criteria

RAC agrees with the Dossier Submitter that the negative result of one modified Buehler test does not negate positive results of three LLNA tests of high reliability, which are supported by three positive *in vitro* tests assessing Key event 2 of the AOP for skin sensitisation. The discrepancy in the results from two types of *in vivo* tests could be due to different batches used in these assays (old FEK-99 blend used in modified Buehler test *vs.* KPIC, MGK and BRA batches used in new LLNA studies) or possibly different species used in the assays (Ko *et al.* 2010). In any case, according to ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a, the murine LLNA is the first-choice method for *in vivo* testing.

Based on the weight-of-evidence approach, and mainly on positive LLNA studies, RAC proposes to classify *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent as **Skin Sens. Cat. 1 (H317 - May cause an allergic skin reaction).**

RAC considers that although the available data do not suggest *Chrysanthemum cinerariaefolium* extract to be a potent skin sensitiser, there is not sufficient data to enable sub-categorisation with confidence.

Supplemental information - In depth analyses by RAC

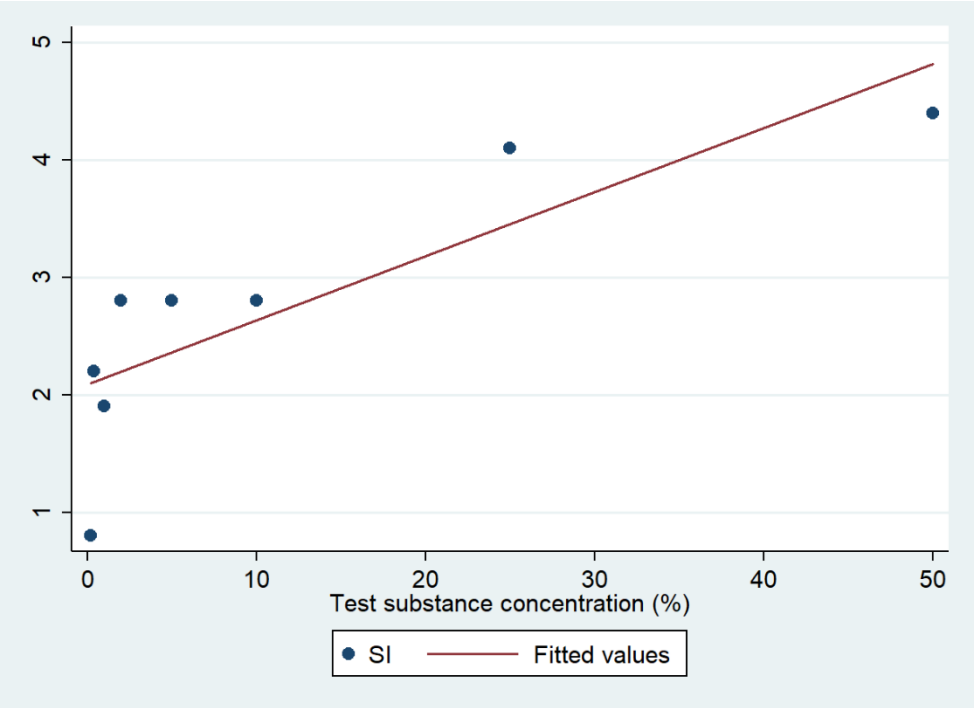
Mean Stimulation Indices observed in LLNA studies with Pyrethrum extracts (copied from the CLH Report)

Test concentration	**5% (v/v) 2.5%**	**10% (v/v) 5%**	**25% (v/v) 12.5%**
Pyrethrum Extract Pale (50% w/w) (KPIC study)	3.5	2.9	3.0

Refined Pyrethrum Concentrate (53.72% w/w) (MGK study)	2.3	4.0	8.1
PY-T-50 Pale Refined Pyrethrins (BRA study)	1.8	6.7	4.3

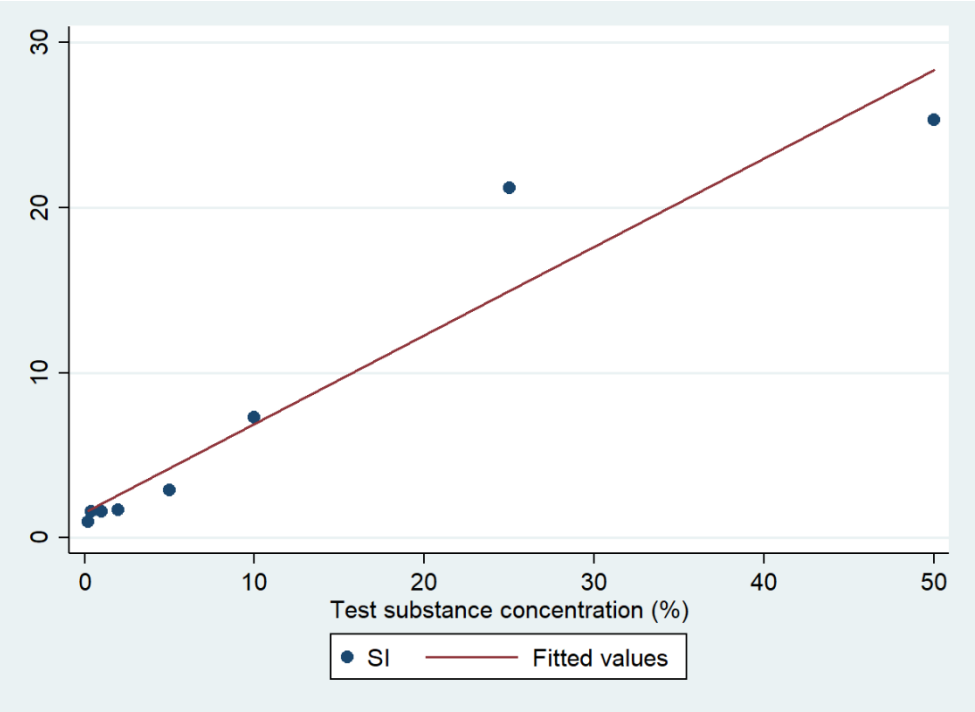
Dose-response curves in *in vitro* studies

a) Study KPIC 2017



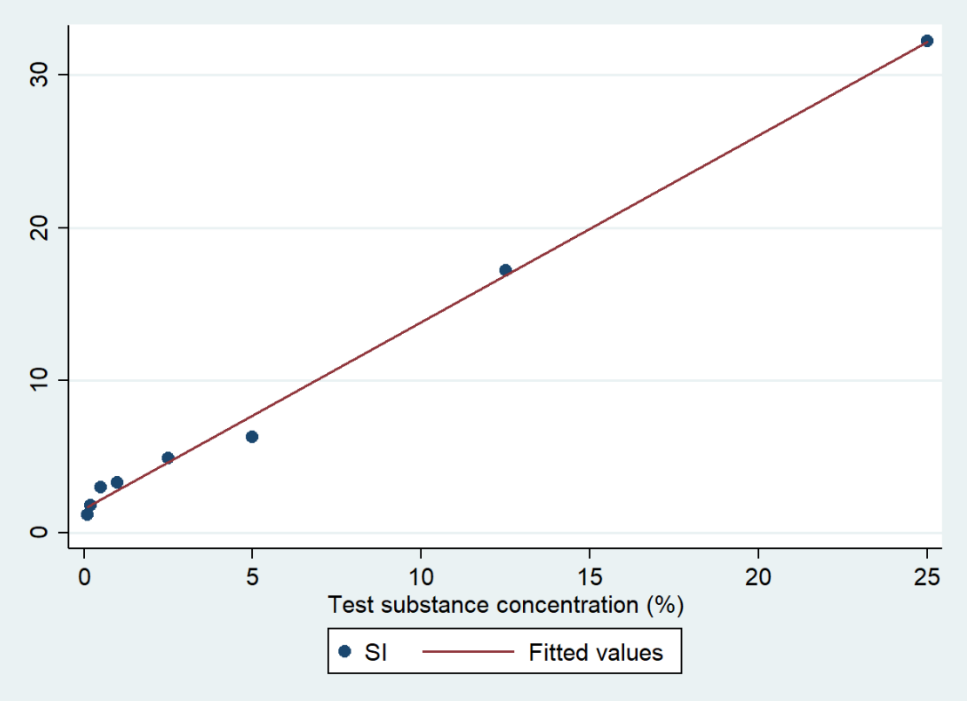
The values on X axis present the concentration of the test substance (which contains 50% pyrethrins) diluted in ethanol: 0.2%, 0.4%, 1%, 2%, 5%, 10%, 25%, and 50%. The percentage of total pyrehtrins at each dose level is, therefore, 0.1%, 0.2%, 0.5%, 1%, 2.5%, 5%, 12.5%, and 25%, respectively. SI = Stimulation index

b) Study MGK 2017



The values on x axis present the concentration of the test substance (which contains 50% pyrethrins) diluted in ethanol: 0.2%, 0.4%, 1%, 2%, 5%, 10%, 25%, and 50%. The percentage of total pyrehtrins at each dose level is, therefore, 0.1%, 0.2%, 0.5%, 1%, 2.5%, 5%, 12.5%, and 25%, respectively. SI = Stimulation index

c) Study BRA 2017



The values on the x axis present the concentration of the test substance (which contains 50% pyrethrins) diluted in ethanol: 0.1%, 0.2%, 0.5%, 1%, 2.5%, 5%, 12.5%, and 25%. The percentage of total pyrehtrins at each dose level is, therefore, 0.05%, 0.1%, 0.25%, 0.5%, 1.25%, 2.5%, 6.25%, and 12.5%, respectively. SI = Stimulation index

A.2.6. Respiratory sensitisation

No data are available.

A2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data are available to indicate that the active substance is a respiratory sensitiser.

A2.6.2 Comparison with the CLP criteria

It does not meet the CLP criteria to be classified as respiratory sensitiser.

A2.6.3 Conclusion on classification and labelling for respiratory sensitisation

Not classified.

A2.6.4 Overall conclusion on respiratory sensitisation related to risk assessment

Based on the available information *Chrysanthemum cinerariaefolium* extract from HCS does not meet the EU criteria to be classified as respiratory sensitiser.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

According to the Dossier Submitter, no data are available to indicate that *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent is a respiratory sensitiser. Therefore, the Dossier Submitter concludes that based on the available information, this substance does not meet the CLP criteria to be classified as respiratory sensitiser.

Comments received during consultation

Due to an oversight, this hazard class was not opened for Consultation.

Assessment and comparison with the classification criteria

This hazard class was not opened for Consultation and therefore, this hazard class was not further discussed by RAC.

A.2.7. Repeated dose toxicity/STOT RE

A.2.7.1. *Short term repeated dose toxicity*

A3.7.1.1 Short-term oral toxicity

Oral repeated dose toxicity

Mouse

In a 2-week toxicity study on mice, Pyrethrum Extract was offered in the diet at concentrations of 5000 and 7000 ppm equivalent to 816 and 1142 mg/kg bw/d total pyrethrins (1250 and 1750 mg/kg bw/d extract) to 50 male animals/treatment group. One animal of the 7000 ppm dosage group died at study day 2. There was no effect on body weight but food consumption was significantly reduced at both dosage levels. A statistically significant increase in absolute and relative liver weights was found at both dosage levels ([redacted]). (KPIC)

Pyrethrum conc. ppm/mg/kg bw/d extract	Clinical signs	Mean bw at day 14 g ± S.D.*	Mean food consumption				Liver weights	
			g/mouse/d		g/kg bw/d			
			week 1	week 2	week 1	week 2	Absolute g	Relative %
0/0	0/15	33 ± 2.5	5.5 ± 0.50*	5.5 ± 0.45	173.8 ± 11.7	163.6 ± 11.4	1.89	5.82
5000/1250	0/50	34 ± 2.5	5.4 ± 0.61	5.5 ± 0.54	164.7 ± 12.3	161.6 ± 10.2	2.82[2)]	8.50[2)]
7000/1750	0/49	34 ± 2.4	5.2 ± 0.41[1)]	5.2 ± 0.38[1)]	162.5 ± 14.3[2)]	155.5 ± 10.1[1)]	2.94[2)]	8.91[2)]

* standard deviation

[1)] significantly different from the control group: $p < 0.05$

[2)] significantly different from the control group: $p < 0.01$

Rat

Pyrethrum extract was offered in three different diets (see above) at dosage levels of 0, 940, 2810, 5640, and 9400 ppm in each diet *ad libitum* for two weeks equivalent to 0, 69, 207, 416, and 672 mg/kg bw/d total pyrethrins (0, 106, 317, 637, and 1030 mg/kg bw/d extract). The groups consisted of 10 male rats each. No mortality and no dose related clinical signs were observed. Animals fed the Purina diet exhibited the largest effect on body weight gain at the 9400 ppm (1030 mg/kg bw/d extract) dosage level with a statistically significant decrease at week 2. The decrease in the other diets was not statistically significant. No clear differences between the three diets were noted. Food consumption depressions paralleled body weight depressions. On the basis of the data obtained, Purina Certified Rodent Chow®# 5002 was selected as the diet to be employed in future studies with pyrethrum extract. 2-week feeding of pyrethrum extract containing diets has comparatively low effects to the rat. The only difference to control animals was found in the highest concentration group (9400 ppm (1030 mg/kg bw/d extract)) which showed less body weight gain in the second week of the test. However, the body weight at the end of week 2 was always higher than at the end of week one ([redacted]). (KPIC)

ES *Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

Parameter	**Control 0 ppm**			**940 ppm 106 mg/kg bw/d extract**			**2180 ppm 317 mg/kg bw/d extract**			**5640 ppm 637 mg/kg bw/d extract**			**9400 ppm 1030 mg/kg bw/d extract**			**+/- dose-response**		
	A*	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
clinical signs	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
body weight [a]	140	139	134	144	146	151	121	133	134	118	132	123	**114**	121	123	-	-	-
observ. period 1[b]	27.4	26	25.5	+ 0.1	+0.4	+1.8	- 2.4	- 1.1	+0.7	- 4.3[2]	- 3.0[2]	- 1.9	- 6.6-[2]	- 3.9[2]	- 2.7[1]			
oserv. period 2 [b]	25.7	24.5	25.0	+ 0.7	+1.2	+1.7	- 1.8	- 0.9	+0.1	- 2.9[1]	- 2.0	- 0.8	**- 2.4[1]**	**- 1.5**	+1.0	-	-	-

* different diets containing pyrethrum in different concentrations

A: Purina Rodent Chow® #5002

B: Ziegler NIH-07 open formula diet

C: Agway Certified Prolab 3200

[a] average changing of weight **(g)** from the beginning of the study to study termination

[b] average changes in food consumption **(g / animal / day)** between two observation periods. difference to control group

[1] significantly different from the control group; $p<0.05$

[2] significantly different from the control group; $p<0.01$

Table A.34 Summary table of oral short-term animal studies (usually 28-day studies)

Summary table of oral short-term animal studies (usually 28-day studies)						
Method, Duration of study, Route of exposure (gavage, in diet, other) Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference
14 days Non-GLP No guideline Reliability 2 Key	Rat Charles River Male 50 per group	Pyrethrum Extract 2 (Lot No. FE K87, FNB 86-2-36A; Purity 54.6%) 0, 940, 2810, 5640, and 9400 ppm total pyrethrins in diet daily; equivalent to 0, 69, 207, 416, and 672 mg/kg bw/d total pyrethrins (0, 106, 317, 637, and 1030 mg/kg bw/d extract)	NOAEL: 5640 ppm (416 mg/kg bw/ d total pyrethrins (637 mg/kg bw/d extract)) LOAEL: 9400 ppm (672 mg/kg bw/ d total pyrethrins (1030 mg/kg bw/d extract))	940, 2810, 5640 ppm (106, 317, and 637 mg/kg bw/d extract): no effects 9400 ppm (1030 mg/kg bw/d extract): body weight gain ↓	-	[redacted] (KPIC) IIIA6.3.1/01

14 days GLP No guideline Reliability 2 Key	Mouse Charles River CD-1 Male 50 per group	Pyrethrum extract 2 (Batch # 011831-00 task force blend # FEK-99Pale; Purity 57.57%) 0, 5000 and 7000 ppm in diet daily; equivalent to 0, 816, and 1142 mg/kg bw/d total pyrethrins (0, 1250, and 1750 mg/kg bw/d extract)	NOAEL: Not determined LOAEL: 5000 ppm (816 mg/kg bw/d total pyrethrins (1250 mg/kg bw/d extract))	5000 ppm (1250 mg/kg bw/d extract): feed intake ↓, liver weight ↑ 7000 ppm (1750 mg/kg bw/d extract): one mortality, feed intake ↓, liver weight ↑	-	[redacted] (KPIC) IIIA6.3.1/02

Value used in the Risk Assessment – Short-term oral toxicity	
Value/conclusion	$AEL_{short\text{-}term}$ 0.2 mg/kg bw/d total pyrethrins (0.31 mg/kg bw/d extract).
Justification for the value/conclusion	Based on the neurotoxicity study in rats given single oral doses, acute neurological disorders and behavioural effects were noted, with a NOAEL of 20 mg/kg bw.

A2.7.1.2 Short-term dermal toxicity

Pyrethrum Extract was administered dermally to five male and five female New Zealand white rabbits in the form of a 25% (w/v) mixture in vegetable oil at doses of 0, 100, 300, or 1000 mg/kg bw once daily of total pyrethrins (0, 153, 460, and 1532 mg/kg bw/d extract), 5 days per week for 3 weeks. Animals in the vehicle control group were given vegetable oil on the same regimen and at the same volume as the group receiving the high dose. One rabbit at 1000 mg/kg bw/d total pyrethrins (1532 mg/kg bw/d extract) was sacrificed in extremis on day 10. Macroscopic examination of this animal did not reveal the cause of death. A low incidence of desquamation and/or red raised areas on the skin at the application site was observed in all groups, including the vehicle controls. Several animals in the treated groups showed very slight to well-defined erythema of the skin at the application site, but no clear pattern with regard to treatment was seen for any of these findings. Microscopic evaluation revealed no evidence of systemic toxicity. The microscopic lesions at the application site included acanthosis, haemorrhage, hyperkeratosis, and chronic inflammation, although haemorrhage was observed only in the group given the vehicle alone. Thus, all of the dermal reactions appeared to be due to the vegetable oil.

The NOAEL for systemic effects was 1000 mg/kg bw/d total pyrethrins (1532 mg/kg bw/d extract), the highest dose tested [redacted]. (KPIC and BRA, MGK and SCJ)

Table A.36 Summary table of dermal short-term animal studies (usually 28-day studies)

Summary table of dermal short-term animal studies (usually 28-day studies)						
Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Dose levels, Surface area, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including target organs)	Remarks (e.g. major deviations)	Reference
EPA 82-2 and OECD 410 21 days GLP Reliability 1 Key	Rabbit New Zealand White rabbits Male/Female 5/sex/group	Pyrethrum extract (Lot FEK-99; Purity 57.574%) 0, 100, 300, and 1000 mg/kg bw/d total pyrethrins (0, 153, 460, and 1532 mg/kg bw/d extract) 5 days /week	NOAEL: 1000 mg/kg bw/d total pyrethrins (1532 mg/kg bw/d extract)	1000 mg/kg bw/d total pyrethrins (1532 mg/kg bw/d extract): 72% animals (and 10-20% of animals treated with lower doses) developed very slight to well defined erytema of the skin at the application syte by day 7. Very slight edema was observed in one female on day 11 and 14. These lessions were partially reversible.	-	[redacted] (KPIC) (BRA, MGK and SCJ) IIIA6.3.2

Table A.37 Summary table of human data on short-term dermal toxicity
No data are available.

A2.7.1.3 Short-term inhalation toxicity
No data are available.

A2.7.1.4 Overall conclusion on short-term repeated dose toxicity related risk assessment

Value used in the Risk Assessment – Short-term repeated dose systemic toxicity	
Value	$AEL_{short\text{-}term}$ 0.2 mg/kg bw/d total pyrethrins (0.31 mg/kg bw/d extract).
Justification for the selected value	Based on the neurotoxicity study in rats given single oral doses, acute neurological disorders and behavioural effects were noted, with a NOAEL of 20 mg/kg bw total pyrethrins (31 mg/kg bw extract).
Proposed classification	Not classified

Value/conclusion used in the Risk Assessment – Short-term repeated dose local effects	
Value/conclusion	Not applicable
Justification for the selected value/conclusion	-
Proposed classification	-

A.2.7.2. *Sub-chronic repeated dose toxicity*

A3.7.2.1 Sub-chronic oral toxicity

Mouse

Groups of 15 male and 15 female Charles River CD-1 mice were offered diets containing 0, 300, 1000, 3000, 10000, and 30000 ppm total pyrethrins over a period of 90 days, equivalent to 0, 47, 160, 460, and 1600 mg/kg bw/d total pyrethrins (0, 72, 245, 705, and 2451 mg/kg bw/d extract) for males and 0, 56, 200, 580, and 1800 mg/kg bw/d total pyrethrins (0, 86, 306, 889, and 2758 mg/kg bw/d extract) for females.

All animals of the highest dosage group died or were sacrificed *in extremis* by study day 10. Four males and two females of the 10000 ppm (2451/2758 mg/kg bw/d extract) dosage group also died within the first two days, with clinical signs that included tremors, pale exposed skin, dilated pupils, altered activity, laboured breathing, cold to touch, moribundity, and hunched posture. No treatment related signs of toxicity were observed in the other test groups. The group mean body weights and food consumption were similar for all groups with surviving animals. The liver was the only target organ found. The absolute weight of the liver and the liver/body weight were statistically significantly increased in males and females at 3000 and 10000 ppm (705/889 and 2451/2758 mg/kg bw/d extract), whereas slight but significant increase of the relative liver weight was also found for males in the 300 and 1000 ppm (72 and 245 mg/kg bw/d extract) test groups. A treatment-related increase in the incidence and/or severity of congestion in the liver was observed in surviving male and female mice at 10000 ppm (2451/2758mg/kg bw/d

extract), and an increased incidence but only mild severity was found in < 15% of investigated animals at 3000 ppm (705/889 mg/kg bw/d extract); at 1000 ppm (245/306 mg/kg bw/d extract), only 2 of 15 mice showed mild congestion of the liver on macroscopic observation. In general, macroscopic congestion was slightly more pronounced in male than in female. An increased incidence of hepatocellular hypertrophy was present in surviving male and female mice at 3000 and 10000 ppm (705/889 and 2451/2758 mg/kg bw/d extract). The NOAEL was 300 ppm, equal to 47 mg/kg bw/d total pyrethrins (72 mg/kg bw/d extract) for males and 56 mg/kg bw/d total pyrethrins for females (86 mg/kg bw/d extract) [redacted] (KPIC)

	Control		**300 ppm pyrethrins 72/86 mg/kg bw/d extract**		**1000 ppm pyrethrins 245/306 mg/kg bw/d extract**		**3000 ppm pyrethrins 705/889 mg/kg bw/d extract**		**10000 ppm pyrethrins 2451/2758 mg/kg bw/d extract**		**30000 ppm pyrethrins**	
	m[a]	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**
Number of animals examined	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**14**	**14**	**3**
Mortality	**0/15**	**0/15**	**0/15**	**0/15**	**0/15**	**0/15**	**0/15**	**0/15**	**0/15**	**1/15**	**1/15**	**12/15**
Clinical signs*	**0/15**	**0/15**	**0/15**	**0/15**	**0/15**	**0/15**	**15/15**	**15/15**	**15/15**	**15/15**	**15/15**	**15/15**
Body weight **(diff. control %)**	**588**	**276**	594 **(-1.0)**	280 **(+1.4)**	569 **(-3.2)**	277 **(+0.4)**	548 **(-6.8)**	267 **(-3.3)**	523 **(-11.1)** [1)]	233 **(-15.6)** [1)]	460 **(-21.8)** [1)]	234 **(-15.2)** [2)]
Food consumption g/animal/day **(diff. control %)**	**28.0**	**18.6**	**28.5 (+1.8)**	18.2 **(-2.2)**	27.5 **(-1.8)**	18.0 **(-3.2)**	26.5 **(-5.4)**	17.3 **(-7.0)**	26.7 **(-4.6)**	15.6 **(-16.1)** [1)]	24.3 **(-15.0)** [2)]	15.9 **(-14.9)** [2)]
Liver												
Organ weight g	**25.06**	**11.12**	**27.21**	**11.6**	**25.53**	**11.91**	**28.8**	**14.0** [1)]	**36.42** [1)]	**17.97** [1)]	**39.71** [1)]	**23.11** [1)]
Gross pathology												
Enlargement	**2/15**	**2/15**	**0/15**	**0/15**	**2/15**	**0/15**	**3/15**	**0/15**	**10/15**	**1/14**	**12/14**	**2/03**
Congestion	**2/15**	**0/15**	**0/15**	**0/15**	**2/15**	**0/15**	**3/15**	**0/15**	**10/15**	**1/14**	**12/14**	**2/03**
Microscopic pathology	**3/10**	**2/10**	**1/10**	**1/10**	**1/10**	**1/10**	**1/10**	**1/10**	**3/10**	**1/10**	**-**	**-**
Kidney												
Organ weight g	**4.59**	**2.4**	**4.75**	**2.33**	**4.59**	**2.36**	**4.98**	**2.4**	**5.53** [1)]	**2.33**	**4.91**	**2.36**

[1)] statistically significant different from the control group $p < 0.01$
[2)] statistically significant different from the control group: $p < 0.05$

[a] number of animals affected/total number of animals

Rat

Groups of 15 male and 15 female Charles River CD rats received diets containing 0, 300, 1000, 3000, 10000, and 20000 ppm total pyrethrins over a period of 90 days,

equivalent to 0, 17, 57, 170, 590, and 1200 mg/kg bw/d total pyrethrins (0, 26, 87, 261, 904, and 1839 mg/kg bw/d extract) for males and 0, 22, 74, 220, 710, and 1400 mg/kg bw/d total pyrethrins (0, 34, 113, 337, 1088, and 2145 mg/kg bw/d extract) for females.

During the first 3 to 7 days one female at the 10000 ppm (1088 mg/kg bw/d extract) dosage level, and one male (904 mg/kg bw/d extract) plus 12 females of the 20000 ppm (2145 mg/kg bw/d extract) dosage level died. All other animals survived. Clinical signs of toxicity were only found at animals from the two highest dosage groups. Signs seen were decreased defecation, tremors, increased respiration rate, increased activity and occasionally convulsions. Most signs only occurred during the first two weeks of the study. Statistically significant decreases in mean body weights were observed during most or all of the study in males and females at 10000 and 20000 ppm (904/1088 and 1839/2145 mg/kg bw/d extract), and statistically significant decreases in mean food consumption were seen during most or all of the study in females at 10000 ppm (1088 mg/kg bw/d extract) and males and females at 20000 ppm (1839/2145 mg/kg bw/d extract) when compared with the respective control groups. Statistically significantly decreased mean values for haematocrit and haemoglobin were found in males at 20000 ppm (1839 mg/kg bw/d extract) and for erythrocytes, haematocrit, and haemoglobin in females at 10000 and 20000 ppm (1088 and 2145 mg/kg bw/d extract). Females at 3000 ppm (337 mg/kg bw/d extract) also showed a slightly decreased mean haemoglobin value. The treatment-related macroscopic findings consisted of enlargement and congestion of the liver in both, males and females, but primarily in males, at 10000 and 20000 ppm (904/1088 and 1839/2145 mg/kg bw/d extract); however, the macroscopic observation could not be confirmed microscopically. The absolute liver weight, the liver/body weight ratio and the liver/brain weight ratio were all statistically significantly increased in males at 10000 and 20000 ppm (904 and 1839 mg/kg bw/d extract) and in females at 3000, 10000 and 20000 ppm (337, 1088, and 2145 mg/kg bw/d extract).

Statistically significant increases of absolute kidney weights were observed at males in the 10000 ppm (904 mg/kg bw/d extract) group only; relative kidney weights were increased at both sexes in the 3000, 10000 and 20000 ppm (261/337, 904/1088 and 1839/2145 mg/kg bw/d extract) dosage group. In microscopic investigations of the kidneys from males small focal or multifocal areas of tubular degeneration and regeneration in the renal cortex were observed. Because of the low incidence of such lesions it cannot be proved if these effects are related to the test substance at all.

The NOAEL was 1000 ppm, equal to 57 mg/kg bw/d total pyrethrins (87 mg/kg bw/d extract) for males and 74 mg/kg bw/d total pyrethrins (113 mg/kg bw/d extract) for females [redacted]. (KPIC and BRA, MGK and SCJ)

Body weights are shown in the table below:

Week of study	**Control (mg/kg bw/d extract)**		**300 ppm (mg/kg bw/d extract)**		**1000 ppm (mg/kg bw/d extract)**		**3000 ppm (mg/kg bw/d extract)**		**10000 ppm (mg/kg bw/d extract)**		**20000 ppm (mg/kg bw/d extract)**	
	Male (0)	**Female (0)**	**Male (26)**	**Female (34)**	**Male (87)**	**Female (113)**	**Male (261)**	**Female (337)**	**Male (904)**	**Female (1088)**	**Male (1839)**	**Female (2145)**
-1	325 ± 12.2	172 ± 7.5	323 ± 13.5	171 ± 7.6	316 ± 13.9	172 ± 7.6	322 ± 13.7	173 ± 7.4	318 ± 14.2	169 ± 6.6	319 ± 14.1	170 ± 7.1
Day 0	340 ± 14.8	182 ± 9.2	338 ± 15.1	182 ± 8.9	332 ± 18.1	183 ± 7.8	335 ± 16.6	181 ± 9.0	331 ± 15.2	176 ± 8.0	330 ± 14.2	177 ± 9.5
1	379 ± 19.0	197 ± 11.6	377 ± 15.0	196 ± 9.8	366 ± 18.0	196 ± 11.0	368 ± 23.5	193 ± 10.7	349[2] ± 19.9	180[2] ± 9.9	296[2] ± 22.6	164[2] ± 13.1

2	403 ± 21.4	205 ± 11.5	404 ± 17.4	207 ± 11.6	390 ± 37.8	208 ± 10.9	386 ± 25.8	206 ± 9.9	372[2] ± 24.1	191[2] ± 8.1	329[2] ± 23.6	187[1] ± 11.0
3	435 ± 22.8	222 ± 15.3	438 ± 22.6	222 ± 14.3	421 ± 47.4	219 ± 11.9	419 ± 29.7	221 ± 9.7	400[2] ± 25.1	203[2] ± 10.7	357[2] ± 24.9	203 ± 2.9
4	457 ± 26.6	229 ± 17.0	461 ± 25.2	232 ± 15.7	445 ± 49.7	230 ± 12.6	440 ± 40.6	225 ± 9.1	421[1] ± 30.7	209[2] ± 9.2	382[2] ± 30.1	213[2] ± 3.5
5	478 ± 28.2	234 ± 18.6	486 ± 23.1	238 ± 17.0	469 ± 48.6	239 ± 14.2	462 ± 40.6	234 ± 12.4	442[1] ± 30.3	216[2] ± 10.6	401[2] ± 29.1	220 ± 3.0
6	501 ± 28.3	243 ± 20.3	510 ± 28.7	249 ± 19.7	489 ± 52.5	251 ± 26.4	478 ± 44.5	242 ± 10.7	459[1] ± 31.5	221[2] ± 9.2	416[2] ± 31.3	228 ± 9.3
7	525 ± 33.6	254 ± 21.9	533 ± 31.9	257 ± 21.1	514 ± 50.0	251 ± 17.4	497 ± 42.8	250 ± 10.4	480[2] ± 38.3	228[2] ± 10.2	431[2] ± 32.3	235[1] ± 7.2
8	533 ± 34.8	257 ± 23.0	541 ± 31.2	261 ± 22.0	519 ± 52.7	256 ± 15.8	502 ± 44.4	249 ± 12.0	485[2] ± 40.3	229[2] ± 8.6	438[2] ± 30.1	235[1] ± 8.7
9	549 ± 34.8	259 ± 23.7	556 ± 33.5	265 ± 20.8	535 ± 57.7	264 ± 17.1	516 ± 44.5	259 ± 12.8	499[2] ± 42.7	232[2] ± 11.4	447[2] ± 32.8	240 ± 7.8
10	561 ± 36.7	269 ± 25.3	571 ± 34.0	272 ± 23.6	547 ± 61.4	269 ± 16.5	530 ± 45.6	264 ± 13.0	508[2] ± 44.3	234[2] ± 10.1	458[2] ± 34.6	244[1] ± 9.7
11	577 ± 37.3	273 ± 25.2	584 ± 41.6	279 ± 24.2	560 ± 64.7	274 ± 17.5	548 ± 49.2	266 ± 12.8	526[1] ± 46.6	236[2] ± 10.5	468[2] ± 34.5	244[1] ± 12.1
12	583 ± 41.8	277 ± 29.0	589 ± 39.1	279 ± 24.2	567 ± 62.2	277 ± 19.4	548 ± 47.8	267 ± 15.1	529[1] ± 48.4	239[2] ± 10.6	468[2] ± 35.9	243[2] ± 12.7
13	588 ± 38.7	276 ± 26.9	594 ± 44.8	280 ± 23.0	569 ± 59.8	277 ± 21.7	548 ± 56.2	267 ± 18.1	523[2] ± 54.1	233[2] ± 12.2	460[2] ± 37.6	234[1] ± 15.0

[1] Significantly different from the control group; $p<0.05$
[2] Significantly different from the control group; $p<0.01$

Parameter	Assessment time	0 ppm (mg/kg bw/d extract)		300 ppm (mg/kg bw/d extract)		1000 ppm (mg/kg bw/d extract)		3000 ppm (mg/kg bw/d extract)		10000 ppm (mg/kg bw/d extract)		20000 ppm (mg/kg bw/d extract)	
		Male (0)	Female (0)	Male (26)	Female (34)	Male (87)	Female (113)	Male (261)	Female (337)	Male (904)	Female (1088)	Male (1839)	Female (2145)
Erythrocytes ($x10^6$/cmm)	Week 13	8.09±0.49	7.55±0.29	7.92±0.43	7.23±0.57	8.02±0.41	7.59±0.31	8.03±0.24	7.28±0.32	7.82±0.48	6.96[2]±0.45	7.59±0.66	6.85[1]±0.27
Haemoglobin (g/dL)	Week 13	17.2±0.62	16.6±0.52	16.8±0.89	16.0±1.17	17.1±0.82	16.5±0.66	17.0±0.56	15.5[2]±0.82	16.4±0.91	14.7[2]±1.44	15.5[2]±1.35	13.3[2]±0.59
Haematocrit (%)	Week 13	50.0±1.76	49.4±1.57	49.6±2.39	47.5±3.55	49.9±2.30	49.4±1.70	49.8±1.44	46.7±2.22	49.3±2.46	45.5[2]±2.94	46.4[1]±4.36	40.7[2]±1.57
MCV (microns^3)	Week 13	62±3.2	66±1.1	63±3.1	66±1.8	62±2.3	65±1.1	62±1.7	64±1.8	63±0.9	65±2.0	61±2.3	59[2]±1.2
MCH (pg)	Week 13	21.3±0.95	22.0±0.45	21.3±0.94	22.2±0.75	21.3±1.00	21.8±0.57	21.2±0.80	21.3[2]±0.61	21.0±0.50	21.1±1.38	20.4±0.75	19.5[2]±0.68
MCHC (g/dL)	Week 13	34.5±0.66	33.6±0.39	33.9±0.52	33.7±0.51	34.2±0.51	33.5±0.44	34.2±0.57	33.2[1]±0.49	33.3[2]±0.62	32.3[1]±1.85	33.3[2]±0.80	32.7±0.57

[1] Significantly different from the control group; p<0.05
[2] Significantly different from the control group; p<0.01

Parameter	Assessment time	0 ppm (mg/kg bw/d extract)		300 ppm (mg/kg bw/d extract)		1000 ppm (mg/kg bw/d extract)		3000 ppm (mg/kg bw/d extract)		10000 ppm (mg/kg bw/d extract)		20000 ppm (mg/kg bw/d extract)	
		Male (0)	Female (0)	Male (26)	Female (34)	Male (87)	Female (113)	Male (0)	Female (0)	Male (26)	Female (34)	Male (87)	Female (113)
Aspartate aminotransferase (IU/L)	Week 13	92±16.1	98±64.5	83±10.8	83±10.3	95±15.4	80±8.5	201±301.8	84±17.1	138±123.0	79±18.0	75[1]±15.6	76±15.0
Alanine aminotransferase (IU/L)	Week 13	37±6.3	49±50.3	31[1]±3.3	31±8.1	37±8.2	29±4.0	194±444.6	32±15.5	206±398.8	42±18.1	55±26.9	45±26.0
Glucose (mg/dL)	Week 13	113±21.0	107±6.9	105±13.4	100±10.3	102±8.3	93[2]±10.1	99±19.3	93[2]±9.7	106±15.8	97±9.2	108±11.9	105±7.5
Urea nitrogen (mg/dL)	Week 13	13.5±1.59	15.0±2.24	13.8±1.58	15.3±1.73	13.4±2.06	16.1±2.12	14.3±2.28	16.5±2.94	17.6[2]±3.60	19.3±7.67	18.1[2]±2.35	19.4±3.25
Creatinine (mg/dL)	Week 13	0.5±0.11	0.6±0.11	0.5±0.07	0.7±0.11	0.5±0.09	0.7±0.07	0.6±0.14	0.7±0.09	0.6±0.08	0.7[1]±0.13	0.6±0.09	0.9[2]±0.12
Total bilirubin (mg/dL)	Week 13	0.2±0.06	0.2±0.07	0.2±0.04	0.2±0.05	0.2±0.05	0.2±0.03	0.1[2]±0.05	0.1[2]±0.05	0.2±0.04	0.2[1]±0.05	0.2±0.00	0.2±0.00
Albumin (g/dL)	Week 13	3.8±0.19	4.1±0.19	3.8±0.24	4.1±0.33	3.9±0.16	4.2±0.16	3.9±0.15	4.2±0.13	4.1[2]±0.22	4.7[2]±0.24	4.4[2]±0.12	4.9[2]±0.21
Globulin (g/dL)	Week 13	3.1±0.18	2.9±0.23	3.1±0.37	2.9±0.22	3.1±0.36	2.8±0.19	3.0±0.25	2.9±0.21	3.0±0.23	2.9±0.22	2.7[2]±0.30	3.1±0.06
Total protein (g/dL)	Week 13	6.9±0.31	7.0±0.37	6.8±0.45	7.0±0.46	6.9±0.37	7.0±0.31	6.9±0.30	7.2±0.23	7.1±0.37	7.6[2]±0.41	7.1±0.38	7.9[2]±0.15

[1]Significantly different from the control group; p<0.05
[2]Significantly different from the control group; p<0.01

Parameter	**0 ppm (mg/kg bw/d extract)**		**300 ppm (mg/kg bw/d extract)**		**1000 ppm (mg/kg bw/d extract)**		**3000 ppm (mg/kg bw/d extract)**		**10000 ppm (mg/kg bw/d extract)**		**20000 ppm (mg/kg bw/d extract)**	
	Male (0)	**Female (0)**	**Male (26)**	**Female (34)**	**Male (87)**	**Female (113)**	**Male (0)**	**Female (0)**	**Male (26)**	**Female (34)**	**Male (87)**	**Female (113)**
Body weight (g)	586±40.2	274±27.9	594±43.3	278±22.1	564± 69.8	273±26.7	549± 57.3	266±16.1	523[2]±56.3	236[2]±10.4	460[2]±38.6	238[1]±16.9
Brain/body weight (% x 10)	3.67±0.378	6.82±0.77	3.57±0.32	6.81±0.65	3.79±0.47	6.99±0.76	3.95±0.51	7.03±0.52	4.06[1]±0.34	7.76[2]±0.45	4.54[2]±0.42	7.97[1]±0.38
Adrenal/body weight (% x 10^3)	11.2±1.62	25.3±4.00	11.7±2.77	26.2±4.49	11.5±2.55	25.4±4.19	11.1±2.78	28.8±4.00	13.1±2.59	28.5±4.37	14.8[2]±3.12	25.4±4.23
Kidney (g)	4.59±0.44	2.27±0.21	4.75±0.41	2.34±0.13	4.59±0.53	2.25±0.20	4.98±0.54	2.40±0.15	5.53[2]±0.51	2.33±0.24	4.91±0.45	2.36±0.37
Kidney/body weight (% x 10)	7.82±0.5	8.32±0.74	8.01±0.56	8.46±0.66	8.19±0.76	8.25±0.63	9.10[2]±0.83	9.04[1]±0.39	10.6[2]±0.70	9.88[2]±0.88	10.7[2]±0.82	9.85[2]±0.84
Kidney/brain weight (% x 10^{-2})	2.15±0.24	1.23±0.13	2.26±0.20	1.25±0.10	2.18±0.29	1.19±0.11	2.33±0.31	1.29±0.09	2.62[2]±0.19	1.28±0.13	2.37±0.22	1.24±0.17
Liver (g)	25.1±3.42	11.1±0.90	27.2±3.93	11.6±1.19	25.5±4.07	11.9±1.57	28.8±4.05	14.0[2]±1.24	36.4[2]±6.20	18.0[2]±1.97	39.7[2]±5.23	23.1[1]±2.84
Liver/body weight (%)	4.26±0.39	4.08±0.33	4.59±0.64	4.18±0.29	4.54±0.54	4.36±0.43	5.25[2]±0.50	5.27[2]±0.49	6.94[2]±0.80	7.61[2]±0.66	8.63[2]±0.80	9.67[2]±0.51
Liver/brain weight (% x 10^{-2})	11.8±1.79	6.04±0.70	12.9±1.93	6.20±0.80	12.1±2.14	6.32±0.95	13.5±2.156	7.54[2]±0.90	17.2[2]±2.54	9.86[2]±1.18	19.2[2]±2.56	12.2[2]±1.25
Testis/body weight (% x 10^{-2})	6.59±0.67	--	6.24±1.08	--	6.67±1.26	--	7.05±0.93	--	6.99±0.77	--	8.21[2]±0.73	--

[1] Significantly different from the control group; $p<0.05$
[2] Significantly different from the control group; $p<0.01$

Dog

In a range-finding study 2 beagle dogs/treatment /sex were offered diets, containing 0, 600, 1000, 3000 and 6000 ppm total pyrethrins for a period of 8 weeks, equal to 0, 18, 30, 86, and 170 mg/kg bw/d total pyrethrins (0, 28, 46, 132, and 261 mg/kg bw/d extract) for males and 0, 19, 29, 94, and 200 mg/kg bw/d total pyrethrins (0, 29, 44, 144, and 306 mg/kg bw/d extract) for females.

One male and both females died or were killed *in extremis* at the 6000 ppm (261/306 mg/kg bw/d extract) dosage level. Treatment related clinical signs were observed in the 3000 and 6000 ppm (132/144 and 261/306 mg/kg bw/d extract) test groups and included inappetence, thin appearance, ataxia, trembling, oily coat, impaired limb function and shallow breathing. Both sexes at 6000 ppm (261/306 mg/kg bw/d extract) lost weight whereas the other test groups did not differ from controls. At the end of the study, in the 3000 ppm and 6000 ppm (132/144 and 261/306 mg/kg bw/d extract) test groups haematocrit, haemoglobin and erythrocyte values were slightly reduced, but there were no other treatment-related haematological findings. Dogs sacrificed *in extremis* (6000 ppm (261/306 mg/kg bw/d extract) group) showed in addition increased values of leukocytes and segmented neutrophils.

Slightly decreased glucose, calcium, phosphorus and cholesterol values were found at the end of the study in males at 6000 ppm (261 mg/kg bw/d extract), and males and females at 3000 ppm (132/144 mg/kg bw/d extract) had slightly decreased cholesterol concentrations. The aspartate and alanine aminotransferase activities of males at 6000 ppm (261 mg/kg bw/d extract) were slightly increased at the end of dosing, and the surviving male at this dose had a very high creatinine phosphokinase value. The absolute weights of the liver in both males and females at 1000 and 3000 ppm (46/44 and 132/144 mg/kg bw/d extract) were increased in a treatment-related fashion, and the absolute weight of the testis at these doses appeared to decrease in a similar manner. The NOAEL was 600 ppm, equal to 18 mg/kg bw/d total pyrethrins (28 mg/kg bw/d extract) for males and 19 mg/kg bw/d total pyrethrins (29 mg/kg bw/d extract) for females ([redacted]). (KPIC and BRA, MGK and SCJ)

Parameter	Assessment time	0 ppm (mg/kg bw/d extract)		600 ppm (mg/kg bw/d extract)		1000 ppm (mg/kg bw/d extract)		3000 ppm (mg/kg bw/d extract)		6000 ppm (mg/kg bw/d extract)	
		Male (0)	Female (0)	Male (28)	Female (29)	Male (46)	Female (44)	Male (132)	Female (144)	Male (261)	Female (306)
Calcium (mg/dL)	Pretest	11.1±0.49	11.4±0.07	11.4±0.14	11.1±0.28	11.3±0.07	11.1±0.00	11.3±0.14	11.2±0.07	11.5±0.21	12.0±0.07
	Week 8	11.8±0.21	11.8±0.14	11.6±0.21	11.5±0.00	11.6±0.21	11.7±0.21	11.6±0.35	11.4±0.21	10.6±0.00	N/A
Phosphorus (mg/dL)	Pretest	7.2±0.78	6.7±0.21	7.7±0.42	6.7±0.07	8.0±1.20	6.3±0.07	7.0±0.42	6.7±0.78	7.5±0.71	6.9±0.78
	Week 8	5.7±0.00	5.4±0.49	5.6±0.14	5.5±0.21	5.8±0.85	5.2±0.07	5.6±0.00	5.0±0.49	5.1±0.49	N/A
Urea nitrogen (mg/dL)	Pretest	17.6±6.51	14.2±2.40	20.6±0.64	15.7±1.70	16.2±4.24	18.0±3.75	16.5±3.54	13.0±1.20	12.5±1.06	15.8±3.68
	Week 8	12.7±1.98	15.5±4.67	16.8±1.70	14.6±0.35	13.1±2.05	15.3±3.39	15.8±0.14	14.6±4.53	18.5±5.30	N/A
Aspartate aminotransferase (IU/L)	Pretest	20±0.7	20±1.4	22±1.4	23±2.1	22±1.4	18±2.1	19±1.4	19±0.0	19±2.1	21±2.8
	Week 8	19±0.0	20±0.7	19±0.7	18±0.0	18±0.0	16±0.0	18±0.7	19±2.8	38±31.8	N/A
Alanine aminotransferase (IU/L)	Pretest	24±3.5	21±1.4	26±2.8	26±1.4	18±2.8	21±3.5	21±3.5	22±4.9	24±0.7	26±4.2
	Week 8	29±2.8	26±0.7	33±2.8	34±2.1	23±1.4	35±2.8	34±0.7	36±2.8	53±28.3	N/A
Creatine phosphokinase (IU/L)	Pretest	88±3.5	95±7.8	118±12.7	112±2.8	106±0.0	84±21.9	95±17.0	100±5.7	96±0.7	100±5.7
	Week 8	103±19.1	72±0.0	95±14.1	87±7.1	99±32.5	61±6.4	81±2.8	80±4.2	426±543.8	N/A
Cholesterol (mg/dL)	Pretest	194±31.1	194±10.6	159±4.2	159±7.8	187±1.4	225±29.7	185±9.2	162±32.5	165±31.1	160±60.8
	Week 8	224±28.3	170±4.2	164±14.1	137±17.0	173±14.1	174±26.2	138±2.8	123±12.0	144±46.7	N/A
Glucose (mg/dL)	Pretest	110±0.7	98±5.7	110±0.0	106±4.9	103±0.7	99±4.9	103±2.8	101±4.9	106±4.2	102±1.4
	Week 8	113±2.8	96±2.1	109±9.2	98±4.2	105±1.4	95±4.2	99±2.8	96±1.4	89±2.1	N/A

Parameter	0 ppm (mg/kg bw/d extract)		600 ppm (mg/kg bw/d extract)		1000 ppm (mg/kg bw/d extract)		3000 ppm (mg/kg bw/d extract)		6000 ppm (mg/kg bw/d extract)	
	Male (0)	Female (0)	Male (28)	Female (29)	Male (46)	Male (0)	Female (0)	Male (28)	Female (29)	Male (46)
Liver (g)	334±2.91	276±7.60	321±15.0	274±52.1	339±30.4	317±67.3	350±54.0	345±2.46	427±0.0	N/A
Testis (g)	16.1±0.24	--	15.1±0.7	--	14.0±0.90	--	12.3±3.90	--	11.7±0.0	--

N/A: not available

Table A.38 Summary table of oral sub-chronic animal studies (usually 90-day studies)

Summary table of oral sub-chronic animal studies (usually 90-day studies)						
Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/ group**	**Test substance (including purity), Vehicle, Dose levels, Duration of exposure**	**NOAEL, LOAEL**	**Results (all dose levels including severity and magnitude of all effects, including also target organs)**	**Remarks (e.g. major deviations)**	**Reference**
OECD TG 408 13 weeks GLP Reliability 1 Key	Rat Charles River CD® Male/Female 15/sex/group	Pyrethrum Extract (Batch # 011831-00 Task force blend # FEK-99; Purity 57.574%) Males: 0, 17, 57, 170, 587, and 1188 mg/kg/d total pyrethrins (0, 26, 87, 261, 904, and 1839 mg/kg bw/d extract) Females: 0, 22, 74, 220, 712, and 1440 mg/kg/d total pyrethrins (0, 34, 113, 337, 1088, and 2145 mg/kg bw/d extract) Daily	NOAEL: 1000 ppm, equal to 57 mg/kg bw/d total pyrethrins (87 mg/kg bw/d extract) for males and 74 mg/kg bw/d (113 mg/kg bw/d extract) for females. LOAEL: 3000 ppm, corresponding to 170 mg/kg bw/d total pyrethrins (261 mg/kg bw/d extract) (males) and 220 mg/kg bw/d total pyrethrins (337 mg/kg bw/d extract) (females).	300 ppm (26/34 mg/kg bw/d extract): No statistically significant effect 1000 ppm (87/113 mg/kg bw/d extract): No statistically significant effect 3000 ppm (261/337 mg/kg bw/d extract): ↓ food consumption, ↓ haemoglobin, ↑ kidney weight, ↑ liver weight in females 10000 ppm and 20000 ppm (904/1088 and 1839/2145 mg/kg bw/d extract): Mortality, ↓ defecation, ↑ respiration rate, tremors, ↑ activity, convulsions, ↓ body weights, ↓ food consumption, ↓ haematological parameters. Liver enlargement and congestion. ↑ Liver weight. ↑ Kidney weight and renal toxicit	-	[redacted] (BRA, MGK and SCJ) IIIA6.4.1/1 [redacted] (KPIC) IIIA6.4.1

Summary table of oral sub-chronic animal studies (usually 90-day studies)

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/ group**	**Test substance (including purity), Vehicle, Dose levels, Duration of exposure**	**NOAEL, LOAEL**	**Results (all dose levels including severity and magnitude of all effects, including also target organs)**	**Remarks (e.g. major deviations)**	**Reference**
OECD TG 408 90-day GLP Reliability 1 Key	Mouse, Charles River CD-1 Male/Female 15/sex/group	Pyrethrum Extract (Batch # 011831-00 Task force blend # FEK-99; Purity 57.574%) 300, 1000, 3000, 10000, 30000 ppm in diet daily *ad libitum*; equivalent to 0, 47, 160, 460, and 1600 mg/kg bw/d total pyrethrins (0, 72, 245, 705, and 2451 mg/kg bw/d extract) for males and 0, 56, 200, 580, and 1800 mg/kg bw/d total pyrethrins (0, 86, 306, 889, and 2758 mg/kg bw/d extract) for females	NOAEL: 300 ppm, corresponding to 47 mgkg bw/d total pyrethrins (72 mg/kg bw/d extract) in male mice and 56 mg/kg bw/d total pyrethrins (86 mg/kg bw/d extract) in female mice LOAEL: 1000 ppm, corresponding to 160 mg/kg bw/d total pyrethrins (245 mg/kg bw/d extract) in male mice and 200 mg/kg bw/d total pyrethrins (306 mg/kg bw/d extract) in female mice	1000 ppm and 3000 ppm (245/306 and 705/889 mg/kg bw/d extract): hepatotoxicity 10000 ppm (2451/2758 mg/kg bw/d extract): mortalities, hepatotoxicity 30000 ppm: all animals died	-	[redacted] (KPIC) IIIA6.4.1/02

Summary table of oral sub-chronic animal studies (usually 90-day studies)						
Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/ group**	**Test substance (including purity), Vehicle, Dose levels, Duration of exposure**	**NOAEL, LOAEL**	**Results (all dose levels including severity and magnitude of all effects, including also target organs)**	**Remarks (e.g. major deviations)**	**Reference**
Compared to OECD TG 409 8 weeks GLP Reliability 1 Key	Dog Beagle dog 2 sex/dose	Pyrethrum Extract (Batch # 011831-00 Task force blend # FEK-99; Purity 57.574%) 600, 1000, 3000, 6000 ppm total pyrethrins in diet daily *ad libitum*; Males: 0, 18, 30, 86, and 170 mg/kg bw/d total pyrethrins (0, 28, 46, 132, and 261 mg/kg bw/d extract) Females: 0, 19, 29, 94, and 200 mg/kg bw/d total pyrethrins (0, 29, 44, 144, and 306 mg/kg bw/d extract)	NOAEL:600 ppm (18 mg/kg bw/d total pyretthrins (28 mg/kg bw/d extract) (males) and 19 mg/kg bw/d (29 mg/kg bw/d extract) (females)) LOAEL: 1000 ppm (30 mg/kg bw/d total pyrethrins (46 mg/kg bw/d extract) (males) and 29 mg/kg bw/day (44 mg/kg bw/d extract) (females))	1000 ppm (46/44 mg/kg bw/d extract): hepatotoxicity, testes weight ↑ 3000 ppm (132/144 mg/kg bw/d extract): clinical signs, haematologic changes hepatotoxicity 6000 ppm (261/306 mg/kg bw/d extract): mortalities	-	[redacted] (KPIC) IIIA6.3.1/03 [redacted] (BRA, MGK and SCJ) IIIA6.4.1/2

Table A.39 Summary table of human data on sub-chronic oral toxicity
No data are available.

A2.7.2.2 Sub-chronic dermal toxicity
No data are available.

A2.7.2.3 Sub-chronic inhalation toxicity

Groups of 15 Sprague Dawley rats of each sex were whole body exposed to analytical concentrations of 0, 11, 30, 100, and 356 mg a.i./m³ for 13 weeks, 6 hours/day, generally 5 days/week and a minimum of 65 exposures in total. Determinations of pesticide size distribution showed an overall mass median aerodynamic diameter of 2.7 µm.

Two animals of the highest concentration group died but only one is considered potentially exposure related.

Clinical signs were observed from 30 mg/m³ onwards and included secretory signs such as nasal discharge and dried material in the facial area in both sexes. In the highest exposure level animals laboured breathing, excess lacrimation, tremors, increased activity and matted coat were also observed. There were no ocular effects.

Body weight gains in the two highest concentration groups were lower than in controls (both sexes). Anaemia by decreased haemoglobin, haematocrit and erythrocyte values was observed from 30 mg/m³ onwards in males and at 356 mg/m³ in females, accompanied with increased leukocyte counts. Significant differences in clinical chemistry parameters were seen primarily in the highest test group, however, the differences were not consistent between sexes. Several significant increases in organ weights or ratios were observed but only the liver weight increases appear to be exposure related. Morphologic abnormalities were observed in the nasoturbinal tissues, nasopharynx, larynx and the lungs. Changes observed in these tissues in all exposure groups, including the air control group, were inflammation, oedema, haemorrhage, emphysema, macrophages, lymphoid cells, mineralisation, glandular dilation and/or goblet cell hyperplasia. These observations were generally graded in the range of minimal to moderate for animals in all exposure groups. The incidence of several other findings indicative of irritation was increased over control. These included squamous/squamoid metaplasia of the pseudostratified columnar or cuboidal epithelium in the larynx and ventral diverticulum. Also, keratinisation of the metaplastic epithelium was observed in the larynx in animals from all treated groups. Morphological abnormalities in the larynx, nasoturbinates, nasopharynx and lungs observed by light microscopy were considered to be localized responses indicative of a treatment-related effect.

The NOAEL for systemic effects was 11 mg a.i./m³ air ([redacted]). (KPIC and BRA, MGK and SCJ)

Parameter	n = 15/sex	0 control	11 (mg/m³)	30 (mg/m³)	100 (mg/m³)	356 (mg/m³)
Leucocytes x 10^3/µl	M	12.10	11.74	12.25	10.54	12.8
	F	8.51	7.9	8.5	8.37	11.25[1)]
Erythrocytes x 10^6/µl	M	8.96	9.04	8.61[1)]	8.60[1)]	8.40[2)]
	F	8.26	8.37	8.41	8.08	8.01

Hematocrit %	M	47.4	47.4	45.3[1)]	45.8	44.7[2)]
	F	45.8	46.1	46.1	44.5	43.3[2)]
Hemoglobin g/dl	M	15.7	15.7	15.1	15.3	14.9
	F	15.4	15.6	15.5	15.1	14.6[2)]

[1)] significantly different from the control group: $p < 0.05$

[2)] significantly different from the control group: $p < 0.01$

Parameter	n = 15/sex	0 control	11 (mg/m³)	30 (mg/m³)	100 (mg/m³)	356 (mg/m³)
AST (GOT) (IU/l)	M	60	63	62	65	61
	F	64	64	63	59	63
ALT (GPT) (IU/l)	M	29	33	29	28	27
	F	29	25	25	21[2)]	23[1)]
Total protein (g/dL)	M	6.5	6.4	6.3	6.2	6.2[1)]
	F	7.0	6.9	6.7	6.7	7.0
Globulin (g/dL)	M	2.5	2.5	2.4	2.3	2.2[2)]
	F	2.2	2.4	2.3	2.2	2.3
Albumin/Globulin ratio (mg/dL)	M	1.6	1.6	1.6	1.8	1.8[1)]
	F	2.3	1.9[1)]	1.9[1)]	2.0	2.1
Glucose (mg/dL)	M	155	169	151	143	148
	F	147	146	157	133	114[2)]
Creatinine (mg/dL)	M	0.5	0.6	0.5	0.5	0.6
	F	0.6	0.6	0.6	0.6	0.7[1)]

AST- Aspartate Amino Transferase (≙GOT = Glutamic Oxaloacetic Transaminase)

ALT - Alanine Amino Transferase (≙GPT = Glutamic Pyruvic Transaminase)

[1)] significantly different from the control group: $p < 0.05$

[2)] significantly different from the control group: $p < 0.01$

Parameter	Air control		10 mg/m³		30 mg/m³		100 mg/m³		350 mg/m³	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Terminal body weight (g)	550±40.8	345±31.6	548±52.3	327±23.0	551±52.7	335±22.1	524±31.9	319*±22.7	520±61.8	307**±22.0
Kidneys organ/body weight (x 1000)	7.47±0.51	7.26±0.52	7.43±0.95	7.70±0.69	7.57±1.10	7.51±0.54	7.77±0.54	7.98**±0.47	8.36*±0.74	8.29**±0.53
Liver weight (g)	14.8±1.57	9.23±0.81	15.0±1.68	8.98±0.82	14.8±1.68	8.98±0.87	14.3±1.49	9.14±0.82	16.4±2.02	11.0**±1.08
Liver organ/body weight (x 100)	2.69±0.15	2.68±0.19	2.73±0.17	2.74±0.19	2.69±0.18	2.68±0.26	2.73±0.19	2.86±0.22	3.16**±0.37	3.57**±0.28
Liver organ/brain (x 1)	6.64±0.70	4.48±0.45	6.81±0.77	4.39±0.44	6.72±0.78	4.25±0.49	6.44±0.84	4.39±0.41	7.41±1.09	5.27**±0.54
Lungs organ/body weight (x 1000)	3.43±0.36	4.14±0.46	3.48±0.54	4.45±0.50	3.33±0.31	4.54±0.46	3.53±0.32	4.55±0.48	3.85*±0.34	4.90**±0.46
Brain organ/body weight (x 1000)	4.07±0.27	6.02±0.59	4.04±0.36	6.28±0.46	4.03±0.33	6.34±0.44	4.27±0.34	6.54*±0.53	4.32±0.63	6.81**±0.52

*, ** Significantly different from control

Table A.40 Summary table of inhalatory sub-chronic animal studies (usually 90-day studies)

Summary table of inhalatory sub-chronic animal studies (usually 90-day studies)						
Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body/ head only), Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference
EPA 82-4 comparable to OECD TG 413 13 weeks GLP Reliability 1 Key	Rat Charles River CD® Male/Female 15/sex/group	Pyrethrum extract (FEK-99; Purity 57.574%) Nominal: 0, 38, 68, 230, 827 mg/m^3 Analytical: 0, 11, 30, 100 and 356 mg/m^3 5 days/week – 13 weeks MMAD = 2.7 ± 1.7 µm Whole body	NOAEL: 11 mg/m^3 LOAEL: 30 mg/m^3	11 mg/m^3: No statistically significant effect 30 mg/m^3: anaemia in males 100 mg/m^3: anaemia, clinical signs (tremors), haematological changes 356 mg/m^3: one mortality, ↑ secretory signs, ↓ body weights, nonregenerative anaemia, changes in clinical chemistry parameters, ↑ liver weights and microscopic changes in the nasopharyngeal tissues, larynx and the lungs.	-	[redacted] (KPIC) (BRA, MGK and SCJ) IIIA6.4.3

Table A.41 Summary table of human data on sub-chronic inhalation toxicity
No data are available.

A2.7.2.4 Overall conclusion on sub-chronic repeated dose toxicity related risk assessment

Value used in the Risk Assessment – Sub-chronic repeated dose systemic toxicity	
Value	$AEL_{medium\text{-}term}$ = 0.14 mg/kg bw/d total pyrethrins (0.22 mg/kg bw/d extract)
Justification for the selected value	Based on a 1-year dog study where hepatic damage was observed at 14 mg/kg bw/d total pyrethrins (22 mg/kg bw/d extract). Since pyrethrins, and therefore *Chrysanthemum* extract, have a rodent-specific hepatotoxic mechanism, it is more appropriate to choose a study conducted without rodents. Sub-chronic study in dogs had only two animals per group since it was a range-finding study, so it is preferable to derive the $AEL_{medium\text{-}term}$ the one-year study in dogs.
Proposed classification	Not classified.

Value/conclusion used in the Risk Assessment – Sub-chronic repeated dose local effects	
Value/conclusion	Not applicable
Justification for the selected value/conclusion	-
Proposed classification	Not classified.

A.2.7.3. *Long-term repeated dose toxicity*

A2.7.3.1 Long-term oral toxicity

Pyrethrum extract was administered to 4 beagle dogs/sex/group for 52 weeks at the following concentrations of actual Pyrethrins: 0, 100, 500, and 2500 ppm equivalent to 0, 2.6, 13.7 and 66 mg/kg bw/d total pyrethrins (0, 3.94, 20.99, and 101.7 mg/kg bw/d extract) for males and 0, 2.8, 14.2 and 75 mg/kg bw/d total pyrethrins (0, 4.35, 21.8, and 114.3 mg/kg bw/d extract) for females. All animals survived to study termination and clinical signs were no remarkable at all dosage levels, although some animals showed some aversion to the 500 and 2500 ppm (20.99/21.8 and 101.7/114.3 mg/kg bw/d extract), dietary concentrations during the first two weeks of the study. No significant differences were observed in mean body weight values between treated and control groups, except for the males at the 100 ppm (3.94 mg/kg bw/d extract) dosage level which gained more weight than the control animals and females at the 2500 ppm (114.3 mg/kg bw/d extract) dose which were slightly less than controls. Some changes and lesions were found in all dose groups; however, these findings were not considered to be treatment related. Those findings are: reduced consumption relative to the controls during the first two weeks of the study; ocular effects; statistically significant differences in parameters such as chloride, cholesterol, urea nitrogen or globulin; macroscopic lesions such as dilatation of the lateral ventricles of the brain and discoloration and mottling of the lungs; microscopic non-neoplastic lesions such as multifocal calcification of the medulla of the kidneys, pituitary cysts, parafollicular cell hyperplasia of the thyroid, dilatation of the lateral ventricles of the cerebrum and multifocal chronic interstitial pneumonia.

Some effects noted at 2500 ppm (101.7/114.3 mg/kg bw/d extract) were considered to be treatment related. These findings were: significant increase in absolute and relative liver weights in males; increase in total leukocytes and segmented neutrophils in females; decrease in values of erythrocytes, haemoglobin and haematocrit in males, although they were not significantly different from the control; increased alanine aminotransferase in females.

The NOAEL for Pyrethrum extract under the conditions of this study is 500 ppm equivalent to 13.7 mg/kg bw/d total pyrethrins (20.99 mg/kg bw/d extract) in males and 14.2 mg/kg bw/d total pyrethrins (21.8 mg/kg bw/d extract) in females [redacted]. (KPIC and BRA, MGK and SCJ)

Parameter	Assessment time	0 ppm (mg/kg bw/d)		100 ppm (mg/kg bw/d)		500 ppm (mg/kg bw/d)		2500 ppm (mg/kg bw/d)	
		Male (0)	Female (0)	Male (3.94)	Female (4.35)	Male (20.99)	Female (21.8)	Male (101.7)	Female (114.3)
Leukocytes (x 10^3 mm^3)	Pretest	8.3 ± 1.77	9.3 ± 1.55	9.1 ± 2.13	8.3 ± 1.65	10.2 ± 1.87	8.4 ± 1.65	8.9 ± 1.93	9.3 ± 1.97
	6	7.7 ± 0.89	9.5 ± 1.91	9.0 ± 1.00	9.3 ± 3.62	10.6 ± 2.34	8.0 ± 1.18	10.0 ± 1.33	10.7 ± 3.73
	12	8.2 ± 0.87	8.6 ± 2.11	8.6 ± 1.92	10.4 ± 2.86	11.4*± 1.74	9.4 ± 2.21	9.2 ± 1.14	17.7* ± 6.23
Segmented neutrophils (x 10^3 mm^3)	Pretest	4.2 ± 0.71	4.7 ± 0.93	5.2 ± 1.80	4.2 ± 1.10	4.5 ± 0.56	4.4 ± 0.97	4.5 ± 1.34	5.0 ± 1.37
	6	4.9 ± 0.74	6.3 ± 1.69	6.1 ± 0.82	6.4 ± 3.43	7.3* ± 1.41	4.9 ± 0.45	6.9 ± 1.42	6.4 ± 2.87
	12	5.3 ± 1.94	4.8 ± 2.43	5.5 ± 1.00	6.0 ± 2.05	6.7 ± 0.36	6.2 ± 2.19	5.3 ± 0.33	12.9* ± 6.02

* Significantly different from the control group; p< 0.05

Parameter	Assessment time	0 ppm (mg/kg bw/d)		100 ppm (mg/kg bw/d)		500 ppm (mg/kg bw/d)		2500 ppm (mg/kg bw/d)	
		Male (0)	Female (0)	Male (3.94)	Female (4.35)	Male (0)	Female (0)	Male (3.94)	Female (4.35)
Chloride (mEq/l)	Pretest	112 ± 1.3	113 ± 3.8	112 ± 3.2	111 ± 1.0	110 ± 2.2	113 ± 0.6	110 ± 3.9	115 ± 2.4
	6	121 ± 1.3	121 ± 2.2	119 ± 2.2	120 ± 2.2	120 ± 3.4	118 ± 2.4	118 ± 2.4	122 ± 2.5
	12	124 ± 1.5	124 ± 5.4	124 ± 2.5	120 ± 3.1	120*± 0.6	121 ± 1.0	123 ± 1.9	123 ± 1.4
Creatine phosphokinase (IU/l)	Pretest	143 ± 37.2	127 ± 46.2	83* ± 8.4	96 ± 20.5	124 ± 12.1	105 ± 30.5	119 ± 64.3	108 ± 46.6
	6	56 ± 16.7	59 ± 6.6	60 ± 6.0	59 ± 17.5	54 ± 15.8	57 ± 9.3	45 ± 5.7	71 ± 23.8
	12	54 ± 20.9	50 ± 10.8	65 ± 14.8	83 ± 44.3	43 ± 10.2	44 ± 4.2	53 ± 27.5	53 ± 11.3
Cholesterol (mg/dl)	Pretest	184 ± 13.0	175 ± 31.5	138 ± 47.5	166 ± 34.2	161 ± 29.2	163 ± 11.1	180 ± 20.3	149 ± 14.4
	6	165 ± 14.8	241 ± 74.3	155 ± 19.2	162 ± 40.6	155 ± 16.9	150* ± 22.0	140* ± 2.2	133* ± 23.9
	12	168 ± 14.2	224 ± 73.1	159 ± 28.8	172 ± 42.3	158 ± 25.5	148 ± 26.2	156 ± 16.2	151 ± 21.2
Alanine aminotransferase (IU/l)	Pretest	30 ± 3.0	29 ± 6.2	27 ± 4.6	28 ± 3.3	32 ± 0.5	28 ± 7.1	27 ± 5.8	27 ± 4.2
	6	32 ± 5.9	29 ± 6.0	29 ± 1.9	25 ± 0.8	34 ± 5.3	28 ± 4.5	40 ± 12.9	37* ± 4.1
	12	43 ± 13.5	28 ± 4.9	34 ± 3.8	26 ± 2.8	54 ± 19.3	29 ± 4.0	52 ± 19.3	39† ± 4.1
Urea nitrogen (mg/dl)	Pretest	15.5 ± 2.14	14.4 ± 2.84	13.3 ± 1.52	15.0 ± 2.95	14.3 ± 3.30	14.3 ± 2.40	16.8 ± 3.43	17.1 ± 3.79
	6	17.4 ± 2.91	17.5 ± 0.99	15.7 ± 0.94	15.1 ± 1.36	14.9 ± 3.60	15.9 ± 1.47	18.7 ± 4.60	18.6 ± 2.17
	12	20.5 ± 2.14	20.9 ± 2.23	16.9 ± 1.71	15.9† ± 1.49	16.7 ± 4.01	17.1* ± 1.69	20.8 ± 5.93	16.2* ± 2.33
Globulin (g/dl)	Pretest	1.6 ± 0.10	2.1 ± 0.10	1.9 ± 0.22	1.8 ± 0.35	1.7 ± 0.15	1.5* ± 0.22	1.6 ± 0.25	1.8 ± 0.26
	6	2.7 ± 0.38	2.4 ± 0.46	3.0 ± 0.51	2.4 ± 0.16	2.6 ± 0.47	2.0 ± 0.37	2.3 ± 0.43	2.3 ± 0.17
	12	3.1 ± 0.33	2.8 ± 0.44	3.3 ± 0.56	3.2 ± 0.34	2.9 ± 0.63	2.3 ± 0.39	2.7 ± 0.28	2.6 ± 0.22

* Significantly different from the control group; p< 0.05
† Significantly different from the control group; p< 0.01

ppm	mg/kg bw/d extract	sex (n = 4)	Body weight (kg)	Liver (g)	Kidney (g)	Pituitary (mg)	Testis (g)	Ovary (g)	Heart (g)
0	0	m	12.9	293.3	54.7	81	17.5		103.6
		f	10.1	238.0	41.3	66		0.93	80.7
100	3.94	m	13.5	306.2	56.7	76	17.7		103.6
	4.35	f	11.0	264.6	45.9	66		1.1	82.2
500	20.99	m	15.9	348.5	61.6	82	18.7		110.5
	21.8	f	10.7	263.0	41.6	78		1.05	79.7
2500	101.7	m	13.1	**381.9**[1)]	61.2	67	18.0		105.6
	114.3	f	9.7	298.2	43.8	66		1.53	83.9

[1)] significantly different from the control group: $p < 0.05$

Table A.42 Summary table of oral long-term animal studies

Summary table of oral long-term animal studies						
Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including target organs)	Remarks (e.g. major deviations)	Reference
EPA 83- 1 comparable to OECD TG 452 52 weeks GLP Reliability 1 Key	Dog Pure bred beagle Male/Female 4/sex/group	Pyrethrum Extract (Batch # 011831-00 Task force blend # FEK-99; Purity 57.574%) Males: 0, 2.57, 13.7, and 66.4 mg/kg/d total pyrethrins (0, 3.94, 20.99, and 101.7 mg/kg bw/d extract) Females: 0, 2.84, 14.2, and 74.6 mg/kg/d total pyrethrins (0, 4.35, 21.8, and 114.3 mg/kg bw/d extract) Daily	NOAEL: 500 ppm, equal to 13.7 mg/kg bw/d total pyrethrins (20.99 mg/kg bw/d extract) for males and 14.2 mg/kg bw/d total pyrethrins (21.8 mg/kg bw/d extract) for females LOAEL: 2500 ppm, corresponding to 66.4 mg/ kg bw/d total pyrethrins (101.7 mg/kg bw/d extract) (males) and 74.6 mg/kg bw/d total pyrethrins (114.3 mg/kg bw/d extract) (females)	100 ppm (3.94/4.35 mg/kg bw/d extract): No treatment related effect 500 ppm (20.99/21.8 mg/kg bw/d extract): No treatment related effect 2500 ppm (101.7/114.3 mg/kg bw/d extract): Treatment related effects;↑ leukocytes, segmented neutrophils and alanine aminotransferase in females; ↓ erythrocytes, haemoglobin and haematocrit: ↑ relative and absolute liver weights in males were recorded.	-	[redacted] (KPIC) IIIA6.4.1/03 [redacted] (BRA, MGK and SCJ) IIIA6.5

Table A.43 Summary table of human data on long-term oral toxicity
No data are available.

A2.7.2.2 Long-term dermal toxicity

No data are available.

A2.7.2.3 Long-term inhalation toxicity

No data are available.

A2.7.3.4 Overall conclusion on long-term repeated dose toxicity related risk assessment

Value used in the Risk Assessment – Long-term repeated dose systemic toxicity	
Value	$AEL_{long\text{-}term}$ = 0.04 mg/kg bw/d total pyrethrins (0.06 mg/kg bw/d extract)
Justification for the selected value	Based on a 2-year rat (carcinogenicity) study.
Proposed classification	Not classified.

Value/conclusion used in the Risk Assessment Long-term repeated dose local effects	
Value/conclusion	Not applicable
Justification for the selected value/conclusion	-
Proposed classification	-

A.2.7.4. *Specific target organ toxicity – repeated exposure (STOT RE)*

A2.7.4.1 Short summary and overall relevance of the provided information on STOT RE

Effects in liver have been observed in one short-term (14 days) repeated dose study by oral exposure in mouse. These effects consisted of a statistically significant increase in absolute and relative weight at 5000 ppm (1250 mg/kg bw/d extract). In two short-term studies by oral exposure in rat and by dermal exposure in rabbit no effects were observed (see A.2.7.1).

Also, effects in liver have been observed in four sub-chronic studies: three studies by oral exposure in mouse, rat and dog and one study by inhalation exposure in rats. These effects consisted of a statistically significant increase in absolute and relative weight, congestion and hepatocellular hypertrophy (see A.2.7.2).

		Liver weight			
Route of exposure	Species	Absolute weight	Relative weight	Congestion	Hepatocellular hypertrophy
Oral	Rat	♂ 904 mg/kg bw/d extract ♀ 337 mg/kg bw/d extract	♂ 904 mg/kg bw/d extract ♀ 337 mg/kg bw/d extract	904/1088 mg/kg bw/d extract	-
	Mouse	♂ 705 mg/kg bw/d ♀ 889 mg/kg bw/d	♂ 705 mg/kg bw/d ♀ 889 mg/kg bw/d	♂ 2451 mg/kg bw/d ♀ 2758 mg/kg bw/d	♂ 705 mg/kg bw/d ♀ 889 mg/kg bw/d
	Dog	46/44 mg/kg bw/d extract	-	-	-
Inhalation	Rat	♂ - ♀ 350 mg/m^3	350 mg/m^3	-	-

In a long-term study by oral exposition in dog an increase in absolute liver weight was statistically significant in males at 101.7 mg/kg bw/d extract (see A.2.7.3).

In two carcinogenicity studies by oral exposure in rat and mouse effects in liver had been observed. In rats, these effects consisted in increased and accentuated lobulations of the liver in males at 1000 ppm, and in increased incidence of hepatic adenoma in females at 3000 ppm. In mice, from 2500 ppm onwards discoloured dark livers and increased absolute and relative liver weights were observed in males and females, and vacuolar fatty changes in the livers of males only (see A.2.9).

Accompanying these effects, an alteration in red cell parameters (hemoglobin, hematocrit and red blood cells) is observed. These effects are considered secondary to hepatotoxicity since they do not occur in the absence of hepatic impairment, except in the case of the inhalation study (where there is no strong dose relationship). Furthermore, they only occur in rats, these effects are not observed in dogs. See table below:

Study	Species	Exposure	Route	Sex	Dose	Effect (blood)	Effect (liver)
[redacted], 1988	Rat	3-month	Oral	♂	0 ppm	No effects	No effects
					300 ppm	No effects	No effects
					1000 ppm	No effects	No effects
					3000 ppm	No effects	↑ relative weight
					10000 ppm	No effects	↑ relative and absolute weights
					20000 ppm	↓ hemoglobin, ↓ hematocrit	↑ relative and absolute weights
				♀	0 ppm	No effects	No effects
					300 ppm	No effects	No effects
					1000 ppm	No effects	No effects
					3000 ppm	↓ hemoglobin	↑ relative and absolute weights
					10000 ppm	↓ hemoglobin, ↓ hematocrit, ↓ erythrocytes	↑ relative and absolute weights
					20000 ppm	↓ hemoglobin, ↓ hematocrit, ↓ erythrocytes	↑ relative and absolute weights
[redacted], 1992			Inhalation	♂	0 mg/m^3	No effects	No effects
					11 mg/m^3	No effects	No effects
					30 mg/m^3	↓ hemoglobin, ↓ hematocrit, ↓ erythrocytes	No effects
					100 mg/m^3	↓ erythrocytes	No effects
					356 mg/m^3	↓ hemoglobin, ↓ hematocrit, ↓ erythrocytes	↑ relative and absolute weights
				♀	0 mg/m^3	No effects	No effects
					11 mg/m^3	No effects	No effects
					30 mg/m^3	No effects	No effects
					100 mg/m^3	No effects	No effects
					356 mg/m^3	↓ hemoglobin, ↓ hematocrit	↑ relative and absolute weights
[redacted], 1988*	Dog	2-month	Oral	♂	0 ppm	No effects	No effects
					600 ppm	No effects	No effects
					1000 ppm	No effects	No effects
					3000 ppm	No effects	↑ relative and absolute weights

					6000 ppm	No effects	↑ relative and absolute weights
				♀	0 ppm	No effects	No effects
					600 ppm	No effects	No effects
					1000 ppm	No effects	↑ absolute weight
					3000 ppm	No effects	↑ relative and absolute weights
					6000 ppm	No effects	Both animals were sacrificed *in extremis*
[redacted], 1990		1-year		♂	0 ppm	No effects	No effects
					100 ppm	No effects	No effects
					500 ppm	No effects	No effects
					2500 ppm	No effects	↑ relative and absolute weights
				♀	0 ppm	No effects	No effects
					100 ppm	No effects	No effects
					500 ppm	No effects	No effects
					2500 ppm	No effects	No effects

*No statistical analysis was performed.

Pyrethrins did not show toxic effects in a two-generation study in rat and in two teratogenicity studies in rat and rabbit (see A.2.10).

A2.7.4.2 Comparison with the CLP criteria

No data are available to indicate that the active substance should be classified for STOT RE. Observed effects are not organ-specific and these are produced at doses higher than those indicated in CLP 3.9.2.9.7. (Table 3.9.3).

A2.7.4.3 Conclusion on classification and labelling for STOT RE

Effects observed in liver are species-specific adaptive responses as explained in A.2.9.1, so *Chrysanthemum cinerariaefolium* extract from HCS does not meet the EU criteria to be classified as STOT RE.

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

Summary of the Dossier Submitter's proposal

Available short-term studies included two oral studies (in rats and mice) and one dermal study (in rabbits). Sub-chronic studies included three 90-day oral studies (in rats, mice, and 8-week study in dogs), a 1-year oral study in dogs, and one 90-day inhalation study in rats.

Only two 14-day oral studies (in rats and mice) were assigned by the Dossier Submitter with a reliability score 2; all other studies were assigned a score of 1. All presented studies were considered by the Dossier Submitter to be key studies.

The Dossier Submitter's short summary on STOT RE

Liver effects have been observed in an oral short-term (14 day) repeated dose study in mice; in four sub-chronic studies (three oral, in mice, rats and dogs, and one inhalation

study in rats); in a long-term oral study in dogs; and in two carcinogenicity studies, in rats and mice.

Changes comprised statistically significant increases in absolute and relative liver weights, congestion and hepatocellular hypertrophy, increased and accentuated lobulations of the liver, increased incidences of hepatic adenoma, discoloured dark livers, and vacuolar fatty changes in the liver.

Alterations in red blood cell parameters (haemoglobin, haematocrit and red blood cells) were considered to be secondary to hepatotoxicity. Furthermore, they were only observed in rats, and not in dogs.

On the other hand, liver effects were not observed in two short-term oral studies in rats and a dermal study in rabbits, nor in a two-generation study in rats and in two teratogenicity studies in rats and rabbits.

The Dossier Submitter considers that the observed effects were not organ-specific; that they are produced at doses higher than those indicated in the CLP Regulation; and that liver effects are species-specific adaptive responses.

Therefore, no classification was proposed for STOT RE.

Comments received during consultation

During targeted consultation in 2023, one MSCA considered that regarding the Applicant's benchmark dose analysis of the incidences of squamous/squamoid metaplasia/hyperplasia in the larynx, several points were identified that should be reconsidered to conclude on classification as STOT RE 2, H373 (inhalation, larynx).

Regarding the Applicant's read-across to similar compounds with repeated neurotoxicity data for pyrethrins, the MS supported the proposal of the dossier submitter for non-classification due to neurotoxicity after repeated exposure because no relevant neurotoxic findings were observed after repeated exposure in the short-term studies available for pyrethrins. The read-across justifications provided was not fully supported due to the limitations in the assessment.

Additional key elements

Neurotoxicity following repeated exposure

A similar rationale to that discussed in the section "*Neurotoxicity and repeated-dose studies"* (below) was employed by the Applicant to justify a lack of repeat dose neurotoxicity study in rodents.

In response to EFSA's request for additional information (within the procedure for renewal of the approval of active substance Pyrethrins), the Applicant performed a read-across analysis to address the missing repeated-dose neurotoxicity endpoint.

The Applicant's read across approach was based on the common insecticidal mode of action (MoA) for pyrethrins and pyrethroids. Pyrethroid insecticides bifenthrin, deltamethrin, alpha-cypermethrin, esfenvalerate, etofenprox, lambda-cyhalothrin and tefluthrin were identified as potential source substances to read across data to pyrethrins. Comparing their toxicity profiles with that of pyrethrins, **deltamethrin** and **tefluthrin** were selected as the most appropriate source substance for read-across, with

available sub-chronic neurotoxicity data. This approach is considered conservative, due to the more potent neurotoxic properties of deltamethrin and tefluthrin compared to pyrethrins.

Currently there are RAC opinions available on the following pyrethroids with STOT RE hazard class assessed:

- Bifenthrin (STOT RE1 Nervous system)
- Cypermethrin (STOT RE2 Nervous system)
- Esfenvalerate (STOT SE1 Nervous system, STOT RE 2 based on mortality)
- Etofenprox (no classification for neuro related effects)
- Tefluthrin STOT RE 1 (Neurotoxicity) proposed by the DS but RAC agreed on no classification for STOT RE due to high acute mortality, classification as Acute Tox. 2 oral and dermal, Acute Tox. 1 inhalation.
- Imiprothin (STOT SE2 Nervous system).

RAC considers that available data do not indicate that repeated dosing of *Chrysanthemum cinerariaefolium* extract would exert more potent neurotoxicity compared to acute exposure. Also, RAC recognises several uncertainties which, at least in the opinion of RAC, preclude using this read-across document to address a missing repeated-dose neurotoxicity endpoint[13]. A non-exhaustive list of these uncertainties is presented below:

- A description of algorithm/rationale by which pyrethroid substances were chosen as potential candidates for read-across is lacking. For example, although for imiprothrin a 90-day oral neurotoxicity study in rats exists (RAC Opinion on imiprothrin, 2018), this substance was not considered by the Applicant. According to ECHA Read-Across Assessment Framework (RAAF) Guideline, criteria for entering the candidate group, or exclusion from the group for a certain substance, should be explained.
- It is not clear why the candidates for read across should represent both type I and II pyrethroids, since pyrethrins belong to the type I group.
- Physico-chemical and toxicokinetic properties of the candidate substances were not included in the evaluation, and it is not explained why they are not considered relevant.
- In the Applicant's report it is not explained in which way the binding potential for the oestrogen and androgen receptors is related to the neurotoxicity of pyrethrins and pyrethroids.
- Finally, two candidate substances for read-across were chosen, but actual read-across for repeated-dose neurotoxicity has not been proposed by the Applicant. From the document it is unclear how the read-across should be performed, especially considering differences in potency and basic structural characteristics of two candidates (primarily regarding the presence of an α-cyano phenoxybenzyl moiety).

<u>Respiratory symptoms in exposed workers - reports on medical surveillance on manufacturing plant personnel</u>

Report 1 (Ogada and Goobe, 1976; cited in the DAR and more information provided by industry during the targeted consultation in 2023). The report is from a radiological

[13] RAAF guideline; ECHA's Advice on using read-across for UVCB substances (May 2022)

(RTG) sand laboratory examination of 58 people who had been employed in a Pyrethrum Extract factory for 1 to 25 years. The high exposure group, comprising 18 people, was subject to daily doses of 6000 ppm of Pyrethrum Extract powder, equivalent to 78 ppm pyrethrins (sum of pyrethrins I and II). The workers were found to be clinically healthy regardless of the degree of exposure to pyrethrins. However, minor pleural lesions of inflammatory type were found in people exposed to high air concentration of Pyrethrum Extract dust. These lesions were reported not to have impaired the workers' health, and the Applicant suggested that they were probably a reaction to irritant dust.

Exposure group **(No of workers)**	**Exposure duration**	**Lung lesions incidence**[1]	**Lung function test**[2]
High exposure (18)	13 workers: 11-22 years 3 workers: 5-6 years 1 worker: 3 years 1 worker: 1 year	**near 100%** (?)[3]	Tested in **13 workers**: 10 high/norm. (77%) 3 low (23%)
Medium exposure (26)	19 workers: 13-25 years 7 workers: 1.5-9 years	**23%** (6/26)	Tested in **15 workers**: 5 high/norm. (33%) 10 low (67%)
Minimal exposure (14)	7 workers: ≥7 years 4 workers: 5-6 years 3 workers: 2-3 years	**0%**	Tested in **7 workers**: 2 high (29%) 5 low (71%)

[1]Pleural thickening, occasional small, and localised calcifications, described as „mild"
[2]Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1)
[3]Incidence was not reported, it was only stated that the changes were "widespread"

Criteria for classifying workers into high, medium, and minimal exposure groups were not described.

Report 2 (Zenz *et al*., 1994; cited in the DAR). This report states that some workers developed sensitivity similar to pollinosis, with sneezing, nasal discharge and nasal stuffiness, and a few cases of asthma due to pyrethrum mixtures have been reported. Some of these workers had a previous history of asthma, with allergies to a wide spectrum of substances. Nevertheless, no serious adverse reactions had been reported.

Assessment and comparison with the classification criteria

Toxicologically relevant findings of short-term and sub-chronic studies in rats, mice, dogs and rabbits are presented in the tables in Appendix I, as well as comparison with the CLP Guidance values for STOT RE. RAC notes uncertainties related to the application of Haber's rule to changes that are compensatory in nature.

The two-generation study in rats and the definitive rat teratology study are not included in the table. In the 2-generation study in rats only decreased body weight and food consumption in dams, and decrease in pups' body weights were observed at 3000 ppm (313 mg/kg bw/day total pyrethrins). Neurotoxic signs of pyrethrin toxicity were not reported, and liver and thyroid toxicity were not adequately investigated in the study.

In the definitive rat teratology study there were no treatment-related mortality, clinical signs, effects on maternal body weight gain, or gross lesions at necropsy.

Summarising the data from the table above, it could be easily identified that the main targets for pyrethrin toxicity are the nervous system and liver.

Comparison with the criteria

Two studies had findings at doses below the GV for STOT RE1:

- In a 90-day inhalation toxicity study in rats, local effects in the upper respiratory tract were observed at doses relevant for classification (further discussed below).
- Tremors were observed in the Range finding study for rat teratology study at a dose below the GV for STOT RE1 (further discussion below).

Six studies had findings at doses below the GV for STOT RE2:

- In a 90-day dietary dose range finding study in mice, only a mild increase in liver weight was observed at a dose below the GV for STOT RE2, and it was without histological any correlate.
- In a 90-day inhalation toxicity study in rats, liver weight changes were observed at doses relevant for classification. However, liver weight changes were not accompanied by histopathological findings or biochemical changes.
- In an 8-week dog study, liver weight was increased by 25% (in females), but was not accompanied by biochemical or histopathological changes. Haematological changes were also observed, but they were of very low magnitude.
- A Range finding study for a rat teratology study showed maternal mortality, convulsions and/or tremors. However, doses relevant for classification at which these effects were observed, were close to or above doses at which lethality started to occur in females in acute oral toxicity studies (around 200 – 400 mg/kg bw).
- The same argument applies for mortality, tremors/convulsions and weight loss observed in the Range finding study for rabbit teratology study.
- In the definitive rabbit teratology study, neurological symptoms were observed already at 104 mg/kg bw/day, and body weight loss at 260 mg/kg bw. Neurotoxic signs are further discussed below, but body weight loss occurred at the dose level at which lethality started to occur in the acute oral toxicity studies.

<u>Liver changes</u>

The only hepatic change observed at doses below the GVs, was an increased absolute and/or relative organ weight, which was not accompanied by adverse biochemical or histopathological changes. At these dose levels, increased liver weight could be, therefore, considered as an adaptive, non-adverse effect.

<u>Neurotoxicity and repeated-dose studies</u>

Neurotoxic symptoms were noted at rather similar dose levels after a single dose in an acute oral neurotoxicity study in rats (66 mg total pyrethrins/kg bw), and in a 10-day teratogenicity range-finding study in rats (78 mg total pyrethrins /kg bw/day) and an 8-week oral study in dogs (90/98 total pyrethrins/kg bw/day). Furthermore, in a 1-year oral dog study no neurotoxic signs were observed, although the highest dose (69/78 mg total pyrethrin/kg bw/day) was just slightly below the dose that produced neurological signs in an 8-week dog study (90/98 total pyrethrins/kg bw/day).

Pyrethrin-induced neurological effects observed in repeated dose toxicity studies were similar or even identical to those observed after acute exposure, and there was no correlation between increase in the incidence or severity of neurotoxic effects and study duration. In some repeated-dose toxicity studies (e.g. a 90-day oral study in rats; an 18-month dietary oncogenicity study in mice), most clinical signs of neurotoxicity occurred only during the first two weeks of the study.

Upper respiratory tract changes in repeated-dose studies

The following questions are raised by the histopathological changes observed in the upper respiratory tract in the 90-day inhalation toxicity study in rats (dose-dependent increases in incidences and severity of squamous/squamoid metaplasia/hyperplasia in the larynx mucosa pseudostratified columnar epithelium and in the larynx ventral diverticulum cuboidal/columnar epithelium): 1) are they toxicologically relevant; 2) are they sufficiently severe to trigger STOT RE classification; 3) if toxicologically relevant and sufficiently severe, which category of STOT RE should be applied?

According to the CLP Regulation and ECHA CLP Guidance, STOT RE is triggered by "significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed", and these are "toxicologically significant changes which have affected the function or morphology of a tissue/organ". Regarding specific organ toxicity, they can include "significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination; multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity; morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver); evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration". Adaptive responses that are not considered toxicologically relevant are not considered relevant for STOT RE classification.

As discussed in the section "RAC evaluation of specific target organ toxicity – single exposure (STOT SE)", in the 90-day inhalation study in rats, (KPIC/MGK, BRA, and SCJ, 1992), although there was no necrosis, fibrosis, or granuloma formation, a dose-dependent increase in the incidence of moderate to moderately severe squamous/squamoid metaplasia/hyperplasia was observed in the larynx mucosa pseudostratified columnar epithelium. An increase in incidence was observed already at the lowest dose tested (0.01 mg/L), while the signs of systemic toxicity were observed only at the highest dose (0.36 mg/L). According to the European Society of Toxicologic Pathologists (ESTP) at the International Expert Workshop on Squamous Metaplasia in the Rodent Larynx in 2006 (Kaufmann *et al.,* 2009), moderate to severe laryngeal squamous metaplasia observed diffusely in multiple levels should be regarded as adverse, since there is a potential for dysfunction of the larynx. In Kaufmann *et al.* (2009) it is also stated that for the assessment of adversity, "it is more relevant to find out whether or not a dysfunction of the organ or tissue can be assumed (e.g., by specifically designed tests for mucociliary clearance), and thus the initially adaptive response has turned into an adverse change." Since in the 90-day inhalation study in rats laryngeal function was not as assessed, and the changes were described as moderate or moderately severe, RAC concludes that these laryngeal changes should be considered as adverse effects related to repeated exposure to pyrethrins.

Human data, although limited, indicate morphological respiratory effects (pleural

thickening, occasional small, localised calcifications) following repeated exposure to pyrethrum extract dust. Lung lesions were described as „mild", they were not related to lung function decline, and in the test their incidences seemed to be correlated with exposure intensity but not with exposure duration.

The CLP guidance value for inhalation of dust/mist/fume is C ≤ 0.02 mg/L/6h/day for STOT RE 1, and 0.02 < C ≤ 0.2 for STOT RE 2. An increased incidence of moderate squamous/squamoid metaplasia/hyperplasia was observed already at 0.01 mg/L in the larynx mucosa pseudostratified columnar epithelium in a 90-day inhalation toxicity study in rats. Nevertheless, moderate squamous/squamoid metaplasia/hyperplasia was observed in the larynx ventral diverticulum cuboidal/columnar epithelium only at the highest dose tested, i.e. 0.36 mg/L. In the occupationally exposed human population, mild morphological changes in the lungs (mild pleural thickening and occasional small, localised calcifications) were observed, but they were not correlated with lung function or general health. Based on these data, classification as **STOT RE 2, H373 (respiratory system, inhalation route)** is considered warranted.

A.2.8. Genotoxicity / Germ cell mutagenicity

A total of six studies was performed. No indication for mutagenic potential of Pyrethrum Extract was observed. No indications for genotoxicity were detected. (KPIC)

A.2.8.1. *In vitro*

In vitro unscheduled DNA synthesis assay (rat primary hepatocytes)

Pyrethrum extract was tested in the unscheduled DNA synthesis assay in male Fischer rat primary hepatocytes with a confirmatory assay. The test article was evaluated at 0.03, 0.1, 0.3, 0.6 and 1.0 µl/ml in both the initial and the confirmatory assay. In a parallel cytotoxicity assay (LDH activity measure) the test article was tested at eight dose levels ranging from 0.003 to 3.0 µL/mL in the initial assay and at seven dose levels ranging from 0.01 to 3.0 µL/mL in the confirmatory assay. The released LDH values from the preliminary cytotoxicity assay indicated that the presence of Pyrethrum extract may have interfered with the LDH assay. Therefore, in this and subsequent cytotoxicity assays, the amount of LDH released from the test article + 1% Triton condition was defined as 100% toxicity. Using this procedure, relative toxicities of 92, 84 and 35% were observed at concentrations of 10, 3.0 and 1.0 µL/mL, respectively. When hepatocyte cultures were examined microscopically, toxicity was also assessed at 10, 3.0, 1.0 and 0.3 µL/mL. At lower concentrations, normal cellular morphology was observed. The results of both the initial and confirmatory UDS assays indicate that none of the test article doses caused significant increase in the mean number of net nuclear grains counts when compared to the appropriate solvent control (DMBA), which induced a significant increase in the average net nuclear counts. Therefore, it is concluded that Pyrethrum extract has not shown any evidence of causing DNA damage in rat liver in this *in vitro* test system (Curren, 1989). (BRA, MGK and SCJ)

In vitro gene mutation study in bacteria (*Salmonella sp.*)

Pyrethrum extract was tested in the *Salmonella*/mammalian-microsome plate incorporation assay using the following bacterial strains: TA98, TA100, TA1535, TA1537 and TA1538. The assay was carried out in the presence and in the absence of an S9 activation system. *Escherichia coli* WP2 uvrA, *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102 were not included in the assay to detect cross-linking mutagens as the guideline recommends. The test article concentrations used in each study with and

without metabolic activation were: 8.8 - 8772 µg/plate in the dose range finding study and 292 - 8772 µg/plate in the mutagenicity and confirmatory assay.

In the dose range-finding study no appreciable toxicity was observed up to 8772 µg per plate. In the mutagenicity assay no positive responses were observed with any of the tester strains in the presence of microsomal enzymes or with tester strains TA100, TA1535 or TA1538 in the absence of microsomal enzymes. Tester strains TA98 and TA1537 exhibited 2.0-fold non-dose responsive increases in the absence of microsomal enzymes. However, non-dose responsive increases are not evaluated as positive.

In the confirmatory assay no positive responses were observed with any of the tester strains either in the presence or the absence of microsomal enzymes. Dose-responsive increases observed with tester strain TA100 in the presence and absence of microsomal enzymes were noted but as they were at less than a 2-fold increase, they were not evaluated as positive. Pyrethrum extract did not cause a positive response with any of the tester stains either in the presence or absence of microsomal enzymes prepared from Aroclor induced rat liver (San & Springfield, 1989). (KPIC and BRA, MGK and SCJ)

***In vitro* cytogenicity study in mammalian cells (CHO-K1)**

An *in vitro* mammalian chromosome aberration test was carried out on Chinese hamster ovary (CHO-K1) cells with the test substance Pyrethrum extract. The test article concentrations used in each study with and without metabolic activation were: 0.03 - 300 µg/ml in the preliminary cytotoxicity assay and 6.25 - 150 µg/ml in the chromosome aberration assay. In the cytotoxicity assay cell growth inhibitions relative to the solvent control were 82% and 100% at 100 and 300 µg/ml in the non-activated test system and 53% and 100% at 100 and 300 µg/ml in the S9-activated test system. The average generation time was delayed from 12.2 to 24 hours at the 100 µg/mL dose level in the non-activated study and from 12.5 to 24 hours at the 100 µg/ml dose level in the S9-activated study. At the 300 µg/ml dose level, complete inhibition of the cell cycle was observed in both the non-activated and the S9-activated test systems. Toxicity (cell growth inhibition) in the chromosome aberration test was between 65% and 100% at concentrations between 50 and 150 µL/mL. Under these concentrations the toxicity was found to be more than 50% cell growth inhibition. The specific data were as follows:

Without S9 mix:

72% cell growth inhibition at 85 µg/ml (12 hours)

78% cell growth inhibition at 100 µg/ml (24 hours)

88% cell growth inhibition at 50 µg/ml (48 hours)

With S9 mix:

65% cell growth inhibition at 70 µg/ml (12 hours)

66% cell growth inhibition at 100 µg/ml (24 hours)

100% cell growth inhibition at 85 µg/ml (48 hours)

No statistically significant increases in chromosome aberrations were observed in the non-activated or S9-activated test systems relative to the solvent control group when cells are harvested at 12, 24 and 48 hours after treatment initiation. Under the conditions of this assay, Pyrethrum extract was concluded to be negative in the chromosome aberration assay using CHO-K1 cells (Curry, 1996). (BRA, MGK and SCJ)

In an almost equal assay, with the only difference being a narrower concentration range (6.25 - 100 µg/mL), the same results were observed (Curry, 1996). (KPIC)

***In vitro* gene mutation assay in mammalian cells (L5178Y)**

Pyrethrin was examined for its potential to induce gene mutations at the Timidine Kinase (TK)-locus of cultured mouse lymphoma L5178Y cells, in both the absence and the presence of a metabolic activation system (S9-mix). The mutant colonies at

concentrations of Pyrethrum extract were scored using the criteria of small and large colonies. Three assays were carried out: Test 1: TK assay with and without metabolic activation at 0.39 - 1200 µg/mL. Test 2: TK assay at 3.0 - 50 µg/mL without metabolic activation, and at 3.0 - 100 µg/mL with metabolic activation. Test 3: TK assay with metabolic activation at 13 - 72 µg/mL. Cytotoxicity was determined in each test measuring the relative total growth (RTG).

The highest concentration of Pyrethrum extract evaluated was 50 µg/mL in the absence of S9-mix and 85 µg/mL with metabolic activation. Pyrethrum extract was cytotoxic to L5178Y cells with and without S9 mix since the RTG was decreased in a dose-related manner above a concentration of 25 µg/mL. At the highest concentrations, the cytotoxicity test indicated a RTG of 6% at 50 µg/mL without metabolic activation and of 3% at 85 µg/mL in the presence of S9-mix. In the mutagenic assay without metabolic activation, the mutant frequency (MF) was not significantly increased at any dose level in either the first or the second assay. However, regarding the first assay, in the presence of S9-mix, the positive control (10 µg/mL MCA) did not comply with the criteria of validation. The positive control and negative control had a similar TK mutant frequency whereas the TK mutant frequency for the positive control should have been higher than 400 TFT-resistant mutants per 10^6 clonable cells and should have been at least twice that of the corresponding negative control. Therefore, this assay was not considered to be valid. About the second assay, in the presence of S9-mix, the mutant frequency was significantly increased at concentration of 85 and 52 µg/mL of Pyrethrum extract, and equivocal response was observed at concentration of 61 and 26 µg/mL. The mutant frequencies were increased by 421, 118, 94 and 59 per 10^6 clonable cells. In general, the amount of small and large colonies was more or less equal. Since 90% cytotoxicity was observed at 85 µg/mL in the presence of S9-mix, the increase in MF may be considered as an artefact. Since 90% cytotoxicity was observed at 85 µg/mL in the presence of S9-mix, the increase in mutant frequency may be considered as an artefact. Hence, the second assay was also not considered to be valid. In the third assay no significant increase of the MF at any dose level was observed. Since no positive and equivocal responses were obtained in the third assay, the findings in the second assay were not of significance.

It was concluded that, under the conditions used in this study, the test substance Pyrethrum extract is not mutagenic at the TK-locus of mouse lymphoma L5178Y cells (Steenwinkel, 2001). (BRA, MGK and SCJ)

In an almost equal assay, three tests were performed at the same concentrations with the only difference being the concentrations of the second test with metabolic activation which are the same as without metabolic activation. Pyrethrum extract did not induce a reproducible significant increase in mutant frequency, neither in the S9-activated nor in the nonactivated system. Also no dose related increase in mutant frequency was observed.

On the basis of this study it is concluded that the test substance Pyrethrum extract is not mutagenic at the TK-locus of mouse lymphoma L5178Y cells (Steenwinkel, 2001). (KPIC)

Further Studies

New GLP-compliant studies on genotoxic activity are provided for two Pyrethrum extracts, Pyrethrum-Extract 50% and Pyrocide 50%. These include *in vitro* gene mutation studies in bacteria (OECD TG 471), *in vitro* cytogenicity studies in mammalian cells (OECD TG 487) and *in vitro* gene mutation studies in mammalian cells (OECD TG 490). These are briefly summarised below.

Microbial Mutagenicity studies (*Salmonella* sp.)

The mutagenic activity of Pyrethrum-Extract 50% and Pyrocide 50% was investigated in reverse gene mutation assays in bacteria according to the testing guideline OECD TG 471. Strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium* and tester strain *E.*

coli WP2 uvrA were exposed to the test items with and without metabolic activation in two independent experiments.

Pyrethrum-Extract 50% (KPIC) was tested with and without metabolic activation at 7 dose levels in experiment I (3.16 - 2500 µg/plate) and experiment II (2 - 2000 µg/plate). In this case, precipitation was noted at concentrations of 1000 µg/plate and above, therefore testing was done only up to 2500 µg/plate in the first and 2000 µg/plate in the second experiment. Pyrocide 50% (MGK) was tested at concentrations of 31.6 – 5000 µg/plate in both experiments with and without metabolic activation. PY-T-50 Pale Refined Pyrethrins (BRA) was tested with and without metabolic activation at 8 dose levels in experiment I (3.16 – 5000 µg/plate) and at 7 dose levels in experiment II (2.0 – 2000 µg/plate).

No mutagenic effect (no increase in revertant colony numbers as compared with control counts) was observed (MF ≤ 1.5%) for any of the test items tested up to the top concentration in any of the 5 test strains in two independent experiments without and with metabolic activation.

All test items (Pyrethrum-Extract 50%, Pyrocide 50%, and PY-T-50 Pale Refined Pyrethrins) were found not to be mutagenic when tested on *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and tester strain *E. coli* WP2 uvrA with or without S9-mix activation and can, therefore, be considered non-genotoxic in this bacterial reverse mutation assay. (KPIC and BRA, MGK and SCJ)

***In vitro* cytogenicity study in mammalian cells (V79)**

In order to investigate the cytogenetic potential in Chinese hamster V79 cells, *in vitro* micronucleus assays following the testing guideline OECD TG 487 were performed with Pyrethrum-Extract 50% and Pyrocide 50%. The selected concentrations were based on the results of a pre-experiment for cytotoxicity. Two independent experiments were carried out for each extract.

With the test item Pyrethrum-Extract 50% (KPIC), experiment I employed an exposure period of 4 h with and without metabolic activation at concentrations of 0.0010 - 0.0050 µL/mL without S9-mix and 0.0125 - 0.050 µL/mL with S9-mix. Experiment II was carried out without metabolic activation at 0.50 - 0.010 µL/mL.

In experiment I without S9-mix, no increase of the cytostasis above 30% was noted at 0.0010 µL/mL. At 0.0025 µL/mL a cytostasis of 31% and at 0.0050 µL/mL a cytostasis of 68% was noted. In experiment I with S9-mix, no increase of the cytostasis above 30% was noted up to 0.0250 µL/mL. At 0.050 µL/mL a cytostasis of 61% was noted. In experiment II without S9-mix, no increase of the cytostasis above 30% was noted up to 0.0050 µL/mL. At 0.075 µL/mL a cytostasis of 32% was noted and at 0.010 µL/mL a cytostasis of 49%. In some cases, although cytostasis > 30%, there are not statistically significant differences with respect to the control group, so under the conditions of this assay, Pyrethrum-Extract 50% was concluded to be negative in the cytogenicity study using V79 cells.

With the test item Pyrocide 50% (MGK), experiment I employed an exposure period of 4 h with and without metabolic activation at concentrations of 0.00010 - 0.010 µL/mL without S9-mix and 0.0125 - 0.10 µL/mL with S9-mix.

Experiment II was carried out without metabolic activation at 0.0005 - and 0.025 µL/mL and 0.010 - 0.10 µL/mL with S9-mix.

In experiment I without S9-mix, no increase of the cytostasis above 30% was noted at 0.0040 µL/mL. At 0.0050 µL/mL a cytostasis of 63% was noted. In experiment I with S9-mix, no increase of the cytostasis above 30% was noted up to 0.050 µL/mL. At 0.060 µL/mL a cytostasis of 51% was noted. In experiment II without S9-mix, no increase of the cytostasis above 30% was noted up to 0.0050 µL/mL. At 0.075 µL/mL a cytostasis of 50% was noted. In experiment I with S9-mix, no increase of the cytostasis above 30% was noted up to 0.040 µL/mL. At 0.065 µL/mL a cytostasis of 48% was noted and at

0.085 µL/mL a cytostasis of 54% was noted. In some cases, although cytostasis > 30%, there are not statistically significant differences with respect to the control group, so under the conditions of this assay, Pyrocide 50% was concluded to be negative in the cytogenicity study using V79 cells.

With the test item PY-T-50 Pale Refined Pyrethrins (BRA), experiment I employed an exposure period of 4 h with and without metabolic activation at concentrations of 0.0025 - 0.0060 µL/mL without S9-mix and 0.025 - 0.10 µL/mL with S9-mix. Experiment II was carried out without metabolic activation at 0.025 - 0.010 µL/mL.

In experiment I without S9-mix, no increase of the cytostasis above 30% was noted at 0.0025 µL/mL. At 0.0040 µL/mL a cytostasis of 44% and at 0.0060 µL/mL a cytostasis of 63% was noted. In experiment I with S9-mix, no increase of the cytostasis above 30% was noted up to 0.050 µL/mL. At 0.10 µL/mL a cytostasis of 51% was noted. In experiment II without S9-mix, no increase of the cytostasis above 30% was noted up to 0.0050 µL/mL. At 0.010 µL/mL a cytostasis of 54% was noted. In some cases, although cytostasis > 30%, there are not statistically significant differences with respect to the control group, so under the conditions of this assay, PY-T-50 Pale Refined Pyrethrins was concluded to be negative in the cytogenicity study using V79 cells.

***In vitro* gene mutation study in mammalian cells (L5178Y)**

The three test items, Pyrethrum-Extract 50%, Pyrocide 50%, and PY-T-50 Pale Refined Pyrethrins were examined for the potential to induce gene mutations at the TK-locus of cultured mouse lymphoma L5178Y cells in both the absence and presence of an S9 metabolic activation system in two independent experiments. The two test items exhibited considerable and similar cytotoxicity with EC50 values in the range of 19 to 56 ng/mL.

Table A.44 Cytotoxicity of the three test items (EC_{50}) and genotoxicity in mouse lymphoma L5178Y cells

	Cytotoxicity EC_{50}		Genotoxicity	
	without S9 (4h) [µg/mL]	with S9 (24h) [µg/mL]	without S9	with S9
Pyrethrum-Extract Pale 50%	0.056	0.056	negative	negative
Pyrocide 50%	0.021	0.037	negative	negative
PY-T-50 Pale Refined Pyrethrins	0.019	0.044	negative	negative

The test item Pyrethrum-Extract Pale 50% (KPIC) was tested in experiment I at concentrations of 0.0025 – 0.12 µL/mL without metabolic activation, and at concentrations of 0.0005 - 0.075 µL/mL with metabolic activation. Experiment II was carried out at concentrations of 0.005 - 0.03 µL/mL without metabolic activation, and at concentrations of 0.02 - 0.085 µL/mL with metabolic activation. In experiments I and II no biologically relevant increase in the number of mutants was found after treatment with Pyrethrum Extract Pale 50% with or without metabolic activation. The Global Evaluation Factor (GEF) was not exceeded (< 126) by the induced mutant frequency at any concentration. Additionally, colony sizing showed no clastogenic effects induced by the test item.

The test item Pyrocide 50% (MGK) was tested in experiment I at concentrations of 0.01 – 0.0375 µL/mL without metabolic activation, and at concentrations of 0.02 - 0.08 µL/mL with metabolic activation. Experiment II was carried out at concentrations of 0.001 - 0.02 µL/mL without metabolic activation, and at concentrations of 0.015 - 0.085 µL/mL with metabolic activation. In experiments I and II no biologically relevant increase in the number of mutants was found after treatment with Pyrocide 50% with or without metabolic activation. The GEF was not exceeded (< 126) by the induced mutant frequency at any concentration. Additionally, colony sizing showed no clastogenic effects induced by the test item.

The test item PY-T-50 Pale Refined Pyrethrins (BRA) was tested in experiment I at concentrations of 0.0025 – 0.035 µL/mL without metabolic activation, and at concentrations of 0.005 - 0.08 µL/mL with metabolic activation. Experiment II was carried out at concentrations of 0.0025 - 0.024 µL/mL without metabolic activation, and at concentrations of 0.01 - 0.075 µL/mL with metabolic activation. In experiments I and II no biologically relevant increase in the number of mutants was found after treatment with PY-T-50 Pale Refined Pyrethrins with or without metabolic activation. The GEF was not exceeded (< 126) by the induced mutant frequency at any concentration. Additionally, colony sizing showed no clastogenic effects induced by the test item.

Under the conditions of these assays following OECD TG 490, Pyrethrum-Extract 50%, Pyrocide 50%, and PY-T-50 Pale Refined Pyrethrins tested up to cytotoxic concentrations in the absence and presence of metabolic activation, did neither induce mutations nor had any chromosomal aberration potential.

Table A.45 Summary table of *in vitro* genotoxicity studies

Summary table of *in vitro* genotoxicity studies					
Method, Guideline, GLP status, Reliability, Key/supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study (e.g. organism (e.g. bacteria), cell type, strains)	Results (including cytotoxicity and +/-S9 mix)	Remarks (e.g. major deviations)	Reference
Bacterial assay gene mutation EPA F, 84-2 GLP Reliability 1 Key	Pyrethrum Extract (Blend FEK-99; Purity 57.574% for KPIC and 57.55% for BRA, MGK and SCJ) 292 585, 877, 2924, 5848, and 8772 µg/plate ± S9	*S. typhimurium* TA98, TA100, TA1535, TA1537 TA 1538	+S9: - -S9: -	Cytotoxicity not evaluated. Non-mutagenic to bacteria	San R.H.C. & Springfield K.A. (1989) (KPIC) (BRA, MGK and SCJ) IIIA 6.6.1
Clastogenicity mammalian cells Chromosomal aberrations EPA F, 84-2 OECD 473 GLP Reliability 1 Key	Pyrethrum Extract Blend FEK-99 Code 96AC14; Purity 55.98% (w/w)) 0, 6.25, 12.5, 25, 40, 50, 55, 70, 85, 100 and 150 µg/mL (BRA, MGK and SCJ) 6.25 - 100 µg/mL (KPIC)	Chinese hamster ovary cells (CHO-K1)	+S9: - -S9: -	Cytotoxicity at 300 µg/mL S-9 activated, at 100 and 300 µg/mL in the non-activated system	Curry P.T. (1996) (KPIC) (BRA, MGK and SCJ) IIIA6.6.2
Clastogenicity mammalian cells Chromosomal aberrations EPA F, 84-2 GLP	Pyrethrum Extract (Blend FEK-99; Purity 57.55%) 0.02 - 0.32 µL/mL	Chinese hamster ovary cells (CHO-K1)	+S9: - -S9: -	Cytotoxicity at the high dose in both the non-activated and the S-9 activated	Putman & Morris (1989) A 6.6.2/02 (KPIC)

Reliability 1 Key				studies	
Mammalian cells gene mutation OECD 476 GLP Reliability 1 Key	Pyrethrum Extract (Blend FEK-99; Purity 57.03%) Preliminary cytotoxicity test: 0, 0.39, 0.78, 1.6, 3.1, 6.2, 12.5, 25, 50, 100, 200, 400, 800 and 1200 µg/mL Mutagenic assay: test 1 without metabolic activation (and with metabolic activation for KPIC): 0, 3.0, 4.3, 6.1, 8.8, 12.5, 17.9, 25.6, 31, 36.5, 40.5, 45 and 50 µg/mL with metabolic activation 0, 3.0, 4.3, 6.1, 8.8, 12.5, 17.9, 25.6, 36.5, 52.2, 61.4, 72, 85 and 100 µg/mL test 2 with metabolic activation: 0, 13, 26, 52, 61 and 72 µg/mL	TK-locus L5178Y cells	+S9: - -S9: -	Cytotoxicity at 26 µg/mL in non-activated systems 52 µg/mL in S9-activated systems	Steenwinkel M-J.S.T. (2001) (KPIC) IIIA6.6.3/01 Steenwinkel M-J.S.T. (2001) (BRA, MGK and SCJ) IIIA6.6.3
Unscheduled DNA synthesis assay EPA 84-2, OECD 482 GLP Reliability 1 Key	Pyrethrum extract (Batch Blend FEK-99; Purity Pyrethrins I = 37.67% (w/w), Pyrethrins II = 19.98% (w/w), total Pyrethrins = 57.55%) Parallel cytotoxicity assay: Eight dose levels ranging from 0.003 to 3.0 µL/mL. Unscheduled DNA synthesis assay: Six dose levels ranging from 0.03 to 1.0 µL/mL.	Primary male rat hepatocyte cell culture	+S9: - -S9: -	As hepatocytes are used, the metabolic activation system is being tested. No increase in the mean number of net nuclear grains count. No DNA damages	Curren R.D. (1989) (BRA, MGK and SCJ) IIIA6.6

In vitro gene mutation study in bacteria OECD 471 EU Method B.13/14 EPA OPPTS 870.5100 GLP Reliability 1 Key	Pyrethrum Extract 50% (Batch number: 2016-5-BB; Purity 50.24%) Pre-experiment: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate Experiment I: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 µg/plate Experiment II: 2.00, 6.32, 20.0, 63.2, 200, 632, 2000 µg/plate	*S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2 uvrA	+S9: - -S9: -	Pyrethrum Extract 50% did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used.	Schreib G. (2016a) (KPIC)
In vitro gene mutation study in bacteria OECD 471 EU Method B.13/14 EPA OPPTS 870.5100 GLP Reliability 1 Key	Pyrocide 50% (Batch No: 10209; Purity 53.72%) Pre-experiment: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 ug/plate Experiment I and experiment II (with and without metabolic activation): 31.6, 100, 316, 1000, 2500 and 5000 µg/plate	*S. typhimurium* TA1535, TA1537, TA98, TA 100 and *E. coli* WP2 uvrA	+S9: - -S9: -	Pyrocide 50% did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used.	Schreib G. (2016b) (MGK)
In vitro gene mutation study in bacteria OECD 471 EC Method B.13/14 EPA OPPTS 870.5100 GLP Reliability 1 Key	PY-T-50 (Batch number: 0116-501-610; Purity 49.35%) Pre-experiment: 3.16, 10.0, 31.6, 100, 316, 100, 316, 1000, 2500, 5000 µg/plate Experiment I: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 µg/plate Experiment II: 2.0, 6.32, 20.0, 63.2, 200, 632, 2000 µg/plate	*S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2 uvrA	+S9: - -S9: -	PY-T-50 did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used.	Schreib G. (2016c) (BRA)
In vitro cytogenicity study in mammalian cells GLP	Pyrethrum Extract 50% (Batch number: 2016-5-BB; Purity 50.24%)	*In vitro* Mammalian Micronucleus Assay V79 Cells	Negative	Pyrethrum Extract 50% did not induce structural	Donath C. (2016a) (KPIC)

Reliability 1 Key	Pre-experiment without S9: 0.0078, 0.0156, 0.0313, and 0.0625 µL/mL Pre-experiment with S9: 0.0078, 0.0156, 0.0313, 0.0625, 0.125, 0.25, and 0.5 µL/mL Experiment I without S9: 0.0010, 0.0025, 0.0040, and 0.0050 µL/mL Experiment I with S9: 0.0125, 0.025, and 0.050 µL/mL Experiment II without S9: 0.0050, 0.0075, and 0.010 µL/mL			and/or numerical chromosomal damage in Chinese Hamster V79 cells.	
In vitro cytogenicity study in mammalian cells GLP Reliability 1 Key	Pyrocide 50% (Batch No: 10209; Purity 53.72%) Pre-experiment without metabolic activation: 0.0078, 0.0156, and 0.0313 µL/mL Pre-experiment with metabolic activation: 0.0078, 0.0156, 0.0313, 0.0625, and 0.125 µL/mL Experiment I without S9: 0.0025, 0.0040, and 0.0050 µL/mL Experiment I with S9: 0.025, 0.050, and 0.060 µL/mL Experiment II without S9: 0.0025, 0.0050, and 0.0075 µL/mL Experiment II with S9: 0.040, 0.065, and 0.085µL/mL	*In vitro* Mammalian Micronucleus Assay V79 Cells	Negative	Pyrocide 50% did not induce structural and/or numerical chromosomal damage in Chinese hamster V79 cells.	Donath C. (2016b) (MGK)
In vitro cytogenicity study in mammalian cells GLP	PY-T-50 (Batch number: 0116-501-610; Purity 49.35%) Pre-experiment without metabolic activation: 0.0078, 0.0156, and	*In vitro* Mammalian Micronucleus Assay V79 Cells	Negative	PY-T-50 Pale Refined Pyrethrins did not induce	Donath C. (2016c) (BRA)

Reliability 1 Key	0.0313 µL/mL Pre-experiment with metabolic activation: 0.0078, 0.0156, 0.0313, 0.0625, and 0.125 µL/mL Experiment I without S9: 0.0025, 0.0040, and 0.0060 µL/mL Experiment I with S9: 0.025, 0.050, and 0.10 µL/mL Experiment II without S9: 0.0025, 0.0050, and 0.010µL/mL			stuctural and/or numerical chromosomal damage in Chinese Hamster V79 cells.	
In vitro gene mutation study in mammalian cells GLP Reliability 1 Key	Pyrethrum Extract 50% (Batch number: 2016-5-BB; Purity 50.24%) Pre-experiment I: 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 1.5, and 2 µL/mL Pre-experiment II without metabolic activation: 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, and 0.12 µL/mL Experiment I: Without metabolic activation: 0.0025, 0.0050, 0.010, 0.025, 0.05, 0.075, 0.1, and 0.12µL/mL With metabolic activation: 0.0005, 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05, and 0.075 µL/mL Experiment II: Without metabolic activation: 0.005, 0.01, 0.014, 0.018, 0.022, 0.024, 0.026, and 0.03 µL/mL With metabolic activation: 0.010, 0.02, 0.04, 0.05, 0.06, 0.07,	Gene mutations (TK-locus) mouse lymphoma L5178Y cells	Negative	Pyrethrum extract 50% is considered to be non-mutagenic in the *in vitro* mammalian cell gene mutation assay (thymidine Kinase locus) in mouse lymphoma L5178Y cells.	Wallner B. (2016a) (KPIC)

	0.075, 0.08, and 0.085 µL/mL				
In vitro gene mutation study in mammalian cells GLP Reliability 1 Key	Pyrocide 50% (Batch No: 10209; Purity 53.72%) Pre-experiment I: 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 1.5, and 2 µL/mL Pre-experiment II without metabolic activation: 0.005, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, and 0.04 µL/mL Experiment I: Without metabolic activation: 0.010, 0.015, 0.020, 0.025, 0.030, 0.0325, 0.035, and 0.0375 µL/mL With metabolic activation: 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.075, and 0.08 µL/mL Experiment II: Without metabolic activation 0.001, 0.002, 0.006, 0.010, 0.014, 0.016, 0.018, and 0.020 µL/mL With metabolic activation 0.015, 0.025, 0.035, 0.045, 0.055, 0.065, 0.075, and 0.08 µL/mL	Gene mutations (TK-locus) mouse lymphoma L5178Y cells	Negative	Pyrocide 50% is considered to be non-mutagenic in the *in vitro* mammalian cell gene mutation assay (thymidine Kinase locus) in mouse lymphoma L5178Y cells.	Wallner B. (2016b) (MGK)
In vitro gene mutation study in mammalian cells GLP Reliability 1 Key	PY-T-50 (Batch number: 0116-501-610; Purity 49.35%) Pre-experiment I: 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 1.5, and 2 µL/mL Pre-experiment II without metabolic activation: 0.001, 0.0025, 0.005, 0.01, 0.02, 0.04, 0.06, and 0.08 µL/mL	Gene mutations (TK-locus) mouse lymphoma L5178Y cells	Negative	PY-T-50 Pale Refined Pyrethrins is considered to be non-mutagenic in the *in vitro* mammalian cell gene	Wallner B. (2016c) (BRA)

	Experiment I: Without metabolic activation: 0.0025, 0.0050, 0.010, 0.020, 0.030, and 0.035 µL/mL With metabolic activation: 0.005, 0.01, 0.02, 0.04, 0.06, and 0.08 µL/mL Experiment II: Without metabolic activation 0.0025, 0.0050, 0.010, 0.016, 0.018, 0.020, 0.022, and 0.024 µL/mL With metabolic activation 0.010, 0.025, 0.050, 0.055, 0.060, 0.065, 0.070, and 0.075 µL/mL			mutation assay (thymidine Kinase locus) in mouse lymphoma L5178Y cells.	

Conclusion used in Risk Assessment – Genotoxicity *in vitro*	
Conclusion	Not genotoxic
Justification for the conclusion	No mutagenic activity was observed for Pyrethrum-Extract 50%, Pyrocide 50%, and PY-T-50 Pale Refined Pyrethrins in a reverse gene mutation assay in bacteria according to the testing guideline OECD TG 471. No biologically relevant increase of the micronucleus frequency was noted in an *in vitro* micronucleus test following OECD TG 487 in Chinese hamster V79 cells. Neither test item induced structural and/or numerical chromosomal damage. When examined for the potential to induce gene mutations at the TK-locus of cultured mouse lymphoma L5178Y cells in both the absence and presence of an S9 metabolic activation system according to OECD TG 490, there was no induction of mutations nor was any chromosomal aberration potential observed. The three test items, Pyrethrum-Extract 50%, Pyrocide 50%, and PY-T-50 Pale Refined Pyrethrins, are considered non-genotoxic based on these new experimental data. The same lack of genotoxic activity was reported for the blend FEK-99 in a battery of *in vitro* tests addressing the same endpoints.

A.2.8.2. *In vivo*

In order to investigate the cytogenetic potential in CFLP mice, *in vivo* micronucleus assay was performed with a mix of 28.4% Pyrethrin I and 25.0% Pyrethrin II. The selected concentrations were based on the results of a dose-finding study. Mice were treated by gavage in two dosages separated by an interval of 24 hours at total dose levels of 0.25, 0.5 and 1.0 mL/kg bw. A negative control group was treated with the vehicle, corn oil, alone. Six hours after the last dose, animals were killed by cervical dislocation and bone marrow smears were analysed microscopically by counting micronuclei in 2000 polychromatic erythrocytes per animal.

There was no increase in the number of micronuclei in treated animals as compared to control group, all counts were within the laboratory standard range for negative controls. In conclusion, the test item was not genotoxic *in vivo*.

Table A6.6.4-1. Summary of micronucleus results in male and female mice[1)]

Dose mL/kg bw	Micronuclei per 2000 polychromatic erythrocytes per animal	
	Mean	**Range**
0	2.3	0 – 5
0.25	1.9	0 – 4
0.5	2.2	0 – 6
1.0	1.6	0 – 4

1) Male and female combined values because there were no sex differences in micronucleus formation

Table A.46 Summary table of *in vivo* genotoxicity studies

Summary table of *in vivo* genotoxicity studies					
Method, duration of study, Guideline, GLP status, Reliability, Key/supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study (e.g. species and strain, sex, no per group, route, frequency of application, sampling times, duration of exposure)	Main effects, Observations (specify regarding dose and sampling time)	Remarks (e.g. major deviations)	Reference
Micronucleus test in rodents Compared to 92/69/EEC (B.12) Non GLP Reliability 2 Key	Pyrethrum Extract (Batch number Refined Pale Concentrate Bulk 99/Pale; Purity 28.4% Pyrethrin I, 25.0% Pyrethrin II) 0.25, 0.5, 1.0 mL/kg bw	CFLP mice (male and female) 5/sex/dose Two with 24 h interval 6 h after last dose Oral (gavage)	No increase in micronuclei	Non-genotoxic	[redacted] (KPIC) IIIA6.6.4

Table A.47 Summary table of human data on genotoxicity

No data are available.

Conclusion used in Risk Assessment – Genotoxicity *in vivo*	
Conclusion	Not genotoxic
Justification for the conclusion	No effects.

A2.8.2.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

A total of seven *in vitro* studies were performed. No indication for mutagenic potential of *Chrysanthemum cinerariaefolium* extract from HCS was observed. No indications for genotoxicity were detected.

In support of this conclusion, the *in vivo* study also shows no evidence of genotoxicity.

A2.8.2.2 Comparison with the CLP criteria

It does not meet the EU criteria to be classified as mutagenic. There are not positive results in tests carried out in mammals and in mutagenicity *in vitro* tests of chromosome aberrations and gene mutation in mammal cells or reverse mutation in bacteria (CLP 3.5.2.2. (Table 3.5.1) and CLP 3.5.2.3.(8.)).

A2.8.2.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified due to inconclusive data.

A2.8.2.4 Overall conclusion on genotoxicity related to risk assessment

Conclusion used in the Risk Assessment – Genotoxicity	
Conclusion	Not genotoxic
Justification for the conclusion	No effects were observed in any of the studies performed.
Proposed classification	Not classified

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In the CLH Report, 13 *in vitro* and one *in vivo* mutagenicity study are described[14]. Out of these, four *in vitro* studies (1st bacterial gene mutation assay, KPIC/BRA, MGK and SCJ, 1989; *In vitro* unscheduled DNA synthesis assay in rat primary hepatocytes, BRA, MGK and SCJ, 1989; *In vitro* mammalian chromosomal aberration test in Chinese hamster ovary (CHO-K1) cells, KPIC/BRA, MGK and SCJ, 1996; *In vitro* gene mutation assay in mammalian cells (L5178Y), KPIC/BRA, MGK and SCJ, 2001), and one *in vivo* study (Micronucleus test in rodents; KPIC, 1976), were evaluated in the DAR (2007).

The Dossier Submitter considered all studies as key studies, and assigned Klimisch score reliability 1 for all *in vitro* studies, and score 2 for *in vivo* micronucleus study from 1976.

***In vitro* studies**

1st bacterial gene mutation assay (KPIC/BRA, MGK and SCJ, 1989)

[14] Additional *in vitro* mammalian chromosomal aberration test in Chinese hamster ovary (CHO-K1) cells from 1989 (KPIC) is mentioned in the CLH Report, but was not further elaborated by the Dossier Submitter.

Chrysanthemum cinerariaefolium extract tested in the Salmonella/mammalian-microsome plate incorporation assay using bacterial strains TA98, TA100, TA1535, TA1537 and TA1538, induced dose-responsive increases with strain TA100 in the presence and absence of microsomal enzymes. However, since less than a 2-fold increase was observed, they were not considered positive. The Dossier Submitter concluded that the test substance did not cause a positive response with any of the tester stains either in the presence or absence of metabolic activation.

2nd, 3rd and 4th bacterial gene mutation assays (KPIC, 2016a; MGK, 2016b; BRA, 2016c)

The mutagenic activity of Pyrethrum-Extract 50%, Pyrocide 50%, and PY-T-50 Pale Refined Pyrethrins, was investigated in GLP and OECD TG-compliant reverse gene mutation assays in bacteria. Since no increase in revertant colony numbers as compared with control counts was observed for any of the test items tested up to the top concentration, the Dossier Submitter concluded that all test items were not mutagenic when tested on *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, and *E. coli* strain WP2 uvrA, with and without metabolic activation in two independent experiments.

In vitro unscheduled DNA synthesis assay in rat primary hepatocytes (BRA, MGK and SCJ, 1989)

The Dossier Submitter considers that the results of both the initial and confirmatory UDS assays, in this GLP and OECD TG-compliant study, indicate that none of the test article doses caused significant increase in the mean number of net nuclear grains counts when compared to the appropriate solvent control. Therefore, the Dossier Submitter concludes that *Chrysanthemum cinerariaefolium* extract has not shown any evidence of causing DNA damage in rat liver in this *in vitro* test system.

In vitro mammalian chromosomal aberration test in Chinese hamster ovary (CHO-K_1) cells (KPIC/BRA, MGK and SCJ, 1996)

In this GLP and OECD TG-compliant test, no statistically significant increases in chromosome aberrations compared to the solvent control group, were observed in Chinese hamster ovary (CHO-K1) cells, either with or without S9-activation, at any harvest time (12, 24 and 48 hours after treatment initiation). The Dossier Submitter concluded that under the assay's conditions, the test substance was negative in the chromosome aberration assay using CHO-K1 cells.

In vitro gene mutation assay in mammalian cells (L5178Y) (KPIC/BRA, MGK and SCJ, 2001)

Pyrethrin extract was examined for its potential to induce gene mutations at the timidine kinase (TK)-locus of cultured mouse lymphoma L5178Y cells, in both the absence and the presence of a metabolic activation system (S9-mix). Three assays were performed, and only in the second assay in the presence of metabolic activation, a statistically significant increase or equivocal results in mutation frequencies were observed. The Dossier submitter concluded that taking into account high cytotoxicity observed at the highest dose tested in the second assay, equivocal results at lower doses in that assay, and the fact that no significant increase was observed in the first and third assay, this study could be considered as negative.

In vitro gene mutation studies in mammalian cells (L5178Y cells) (KPIC, 2016a; MGK, 2016b; BRA, 2016c)

The three test items, Pyrethrum-Extract 50%, Pyrocide 50%, and PY-T-50 Pale Refined Pyrethrins, were examined for the potential to induce gene mutations at the TK-locus of cultured mouse lymphoma L5178Y cells in both the absence and presence of S9 metabolic activation system in two independent experiments, in which considerable cytotoxicity was observed (EC_{50} values ranged from 19 to 56 ng/mL in the absence of metabolic activation, and from 37 to 56 ng/mL in the presence of metabolic activation). The Dossier Submitter summarised that under the conditions of these assays, which are GLP and OECD TG-compliant, three test items neither induced mutations nor had any chromosomal aberration potential, either in the absence or presence of metabolic activation.

In vitro cytogenicity studies in mammalian cells (MN) (KPIC, 2016a; MGK, 2016b; BRA, 2016c)

The three test items, Pyrethrum-Extract 50%, Pyrocide 50%, and PY-T-50 Pale Refined Pyrethrins, were examined for the cytogenetic potential in Chinese hamster V79 cells, in an *in vitro* micronucleus assay, with and without metabolic activation. Selected concentrations were based on the results of a pre-experiment for cytotoxicity. Negative results were found in two independent experiments carried out for each extract. The Dossier Submitter concluded that the test items did not induce structural and/or numerical chromosomal damage in Chinese Hamster V79 cells.

In vivo study

Micronucleus test in rodents (KPIC, 1976)

An *in vivo* micronucleus assay was performed in CFLP mice with a mix of 28.4% Pyrethrin I and 25.0% Pyrethrin II. The selected concentrations were based on the results of a dose-finding study. Mice were treated by gavage in two dosages separated by an interval of 24 hours at total dose levels of 0.25, 0.5 and 1.0 mL/kg bw. A negative control group was treated with the vehicle, corn oil, alone. Six hours after the last dose, animals were killed by cervical dislocation and bone marrow smears were analysed microscopically by counting micronuclei in 2000 polychromatic erythrocytes per animal.

There was no increase in the number of micronuclei in treated animals as compared to control group, all counts were within the laboratory standard range for negative controls. The Dossier Submitter concludes that the test item was not genotoxic *in vivo*.

Overall conclusion

Based on the results from described *in vitro* and *in vivo* studies, the Dossier Submitter concluded that there was no indication for mutagenic or genotoxic potential of *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent and proposed no classification for germ cell mutagenicity.

Comments received during consultation

No comments were received regarding this hazard class.

Assessment and comparison with the classification criteria

In the CLH Report, 13 *in vitro* and one *in vivo* genotoxicity study were described. One additional *in vitro* study is listed in the Table A.45 Summary table of *in vitro* genotoxicity studies. It was an *in vitro* mammalian chromosomal aberration test in Chinese hamster ovary (CHO-K_1) cells (KPIC, 1989), which showed a negative result, but it was not further elaborated in the text. In the DAR and RAR, it is stated that the study, although GLP-compliant and in line with EPA Guideline 84-2 (similar to OECD 473, 1983), is not acceptable because the range of exposures in the main assay was too narrow, and it does not include non-cytotoxic doses and adequate evidence that cells were in M1 at the single harvest time. Also, the positive control without metabolic activation (triethylenemelamine) used in the study is not among those recommended by the guideline. This study, therefore, was not further discussed by RAC.

In response to EFSA's request for additional information (within the procedure for renewal of the approval of active substances, i.e. Pyrethrins), the Applicant provided the following:

- data on cytotoxicity and precipitation at selected doses in pre-experiment for the bacterial gene mutation assays (KPIC, 2016a; MGK, 2016b; BRA, 2016c);
- numerical data for the types of chromatid and chromosome aberrations in an *in vitro* mammalian chromosomal aberration test in Chinese hamster ovary (CHO-K1) cells (KPIC/BRA, MGK and SCJ, 1996);
- historical control data for the use of ethylmethanesulfonate as a positive control substance in an *in vitro* micronucleus assays in Chinese hamster V79 cells (KPIC, 2016a; MGK, 2016b; BRA, 2016c);
- a new *in vivo* micronucleus assay in rodents (Micronucleus test in bone marrow cells of the rat with Pyrocide 50%, KPIC, MGK, BRA, 2022); and
- an *in silico* (Derek Nexus) prediction for photomutagenicity.

In vitro studies

Out of 13 acceptable *in vitro* studies, there are:

- 4 **bacterial gene mutation assays** (out of which one has been described in the DAR, while the other were performed later, i.e. in 2016);
- one **unscheduled DNA synthesis** assay in rat primary hepatocytes;
- one *in vitro* **mammalian chromosomal aberration test** in Chinese hamster ovary (CHO-K_1) cells (from 1996);
- 4 gene **mutation tests at the TK-locus** of mouse lymphoma L5178Y cells (one from 2001, described in the DAR, and three were performed later, i.e. in 2016);
- 3 *in vitro* mammalian **micronucleus assays** in V79 cells (not described in the DAR since they were performed in 2016).

<u>Bacterial gene mutation assays</u>

1st bacterial gene mutation assay (KPIC/BRA, MGK and SCJ, 1989)

This GLP assay was performed with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with and without S9 activation system (prepared from Aroclor 1254 induced rat liver). The assay was performed according to the OECD TG

471, with the exception that the strains *E. coli* WP2 uvrA, *E.coli* WP2 uvrA (pKM101) or *S. typhimurium* TA102, which are able to detect cross-linking mutagens, were not included in the assay.

Based on the range-finding study, dose levels of pyrethrum extract (FEK-99 blend; purity 57.6%) of 292 – 8772 µg/plate (concentrations are not corrected for purity) were applied both in the main mutagenicity and confirmatory assays. No appreciable toxicity was observed at up to 8772 µg/plate of extract (5048 µg/plate for the sum of Pyrethrins I and Pyrethrins II)[15]. Adequacy of exposure concentrations were demonstrated by precipitation of the test material. Both with and without metabolic activation, negative control (vehicle, i.e. acetone) did not show a positive response, and an adequate positive response was observed in appropriate positive controls.

In contrast to the Dossier Submitter's conclusion, RAC considers that the results show a **positive response for TA100**, with and without metabolic activation. The main mutagenicity and confirmatory assays showed a dose-response with an increase up to 1.7 compared to vehicle control (study results are presented in "Supplemental information - In depth analyses by RAC", background document). The Dossier Submitter noted a dose-response relationship for that strain, but since the increase was less than 2-fold, they did not consider this result to be positive. RAC, nevertheless, points out that the need for a 2-fold increase has been questioned, especially for strains with relatively high background reversion frequencies, such as Salmonella strains TA100, TA97, and TA102, in which this cut off value can lead to a false negative result (Cariello and Piegorsch 1996; Mortelmans and Zeiger 2000).

Other strains showed a **negative result**: no dose-response and no reproducible increase at one or more concentrations in the number of revertant colonies per plate were observed.

In contrast to the DS, RAC, therefore, considers that this study indicates a weak mutagenic response of the tested substance.

2nd, 3rd and 4th bacterial gene mutation assays (KPIC, 2016a; MGK, 2016b; BRA, 2016c)

These GLP assays were performed with *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and Escherichia coli strain WP2 uvrA, with and without S9 activation. The assays were performed according to OECD TG 471.

Test substances were Pyrethrum Extract 50% (batch number: 2016-5-BB; purity 50.24%) in the KPIC study, Pyrocide 50% (batch number: 10209; purity 53.72%) in the MGK study, and PY-T-50 (batch number: 0116-501-610; purity 49.35%) in the BRA study.

In all studies there was a pre-experiment and two main experiments. In the main experiments, concentrations of the test substance ranged from 3.16 to 2500 µg/plate in the KPIC and BRA studies, and from 3.16 to 5000 µg/plate in the MGK study.

In the 5 test strains in two independent experiments, without and with metabolic

[15] Pyrethrins I group include pyrethrin 1, cinerin 1, and jasmolin 1, and Pyrethrins II group consists of pyrethrin 2, cinerin 2 and jasmolin 2.

activation, no mutagenic effect (i.e. no increase in revertant colony numbers as compared to the vehicle control) was observed (Mutation Factor (MF)[16] was ≤ 1.5%) up to the top concentration tested, for all three test items (Pyrethrum Extract 50%, Pyrocide 50%, and PY-T-50).

RAC notes that numerical data on revertant colonies are not available to RAC. Nevertheless, as a response to EFSA's request for additional information regarding cytotoxicity and precipitation at selected doses, numerical data (MFs) are shown for TA98 and TA100 in the pre-experiment (although it was not stated which batch was used), and the results were clearly negative: no dose-response relationship was observed and the MFs were up to 1.3. Information on cytotoxicity and precipitation (solubility) provided in the same response justify the selection of test substance concentrations used in the main experiments.

<u>In vitro unscheduled DNA synthesis assay in rat primary hepatocytes (BRA, MGK and SCJ, 1989)</u>

This is a GLP compliant study, performed in line with OECD TG 482. Pyrethrum extract (Blend FEK-99; purity 57.55%) was tested in rat primary hepatocytes in:

- A preliminary cytotoxicity test (dose levels ranged from 0.0003 to 10 µL/mL);
- two trials assessing unscheduled DNA synthesis (Trial II was a confirmatory assay) (dose levels ranged from 0.03 to 3.0 µL/mL, i.e. 0.03, 0.1, 0.3, 0.6, 1.0 and 3.0 µL/mL) using ^{3}H-TdR autoradiography; and
- two parallel cytotoxicity assays (dose levels ranged from 0.003 to 3.0 µL/mL in Trial I, and from 0.01 to 3.0 µL/mL in Trial II).

Cytotoxicity assays showed that dose level of 3.0 µL/mL could not be evaluated for UDS due to excessive toxicity (measured by LDH release from the exposed cells).

In the first UDS assay (Trial I), the mean number of net nuclear grains counts raised from 1.4 ± 2.5 in solvent control to 1.9 ± 3.2 at 1.0 µL/mL dose level (the highest dose at which cytotoxicity assays did not show excessive toxicity). However, the increase was not statistically significant, cell survival at this dose level was 23%, and there was no dose-response relationship in the assay. No such increase was observed in the confirmatory assay (Trial II), and there was no dose-response in this assay as well (study results are available in the DAR, Tables B.6.71 and B.6.72).

Negative and positive controls were appropriately chosen and showed an appropriate response in the study.

RAC agrees with the Dossier Submitter that this *in vitro* test does not indicate that the test substance induces DNA damage in rat liver cells.

<u>In vitro mammalian chromosomal aberration test in Chinese hamster ovary (CHO-K_1) cells (KPIC/BRA, MGK and SCJ, 1996)</u>

This is a GLP study, performed according to OECD TG 473. CHO-K_1 cells cultured *in vitro* were exposed to pyrethrum extract (FEK-99 blend; purity: 55.98%) using dimethylsulfoxide (DMSO) as the solvent. The preliminary cytotoxicity (and solubility)

[16] Mutation Factor: mean number of revertants on the test item plate / mean number of revertants on the vehicle control plate

test was conducted in the absence and presence of the S-9 activation system (at nine concentrations of test article, from 0.03 to 300 µg/mL).

The chromosome aberration assay was conducted in the absence and presence of an Aroclor-induced S-9 activation system at dose levels ranging from 6.25 to 150 µg/mL. The cell harvest times were adjusted to 24 and 48 hours, based on the cell cycle delay observed, and an additional harvest time of 12 hours was also included. Minimally 200 metaphase spreads were examined and scored. Adequate negative and positive controls were included and showed appropriate responses. Statistical analysis of the percent aberrant cells was performed using the Fisher´s exact test for pairwise comparisons (treated groups vs. solvent control), and in the case of a positive Fisher´s test at any test article dose level, the Cochran-Armitage test was used to analyse the dose-response.

Marked toxicity was observed at the highest doses in both the non-activated and S-9 activated studies. The percentage of cells with structural aberrations did not significantly increase in the test group compared to control group at 12, 24, and 48h after treatment initiation, both in the absence and presence of metabolic activation (study results are available in the DAR, Table B.6.67).

Data provided as the additional information in response to EFSA's request showed a slight increase in the number of chromatid aberrations (gaps, breaks, and exchanges), e.g. 2.5 chromatid gaps vs. 0.5 in vehicle control at the 12h harvest and 3.0 vs. 0 at the 48h harvest in the presence of metabolic activation; 2.5 chromatid gaps vs. 0 in vehicle control at the 24h harvest in the absence of metabolic activation; 2.5 chromatid breaks vs. 0 in vehicle control at the 24h harvest in the presence of metabolic activation; 4.5 chromatid exchanges vs. 0 in vehicle control at the 48h harvest in the presence of metabolic activation (data are expressed as average for two analysed flasks per dose level; statistical analysis was not presented) (study results are presented in "Supplemental information - In depth analyses by RAC", background document). Nevertheless, these aberrations occurred only at the highest doses at which high cytotoxicity was observed (approximately 60-80% reduction in the mitotic index compared to vehicle control)[17].

RAC, therefore, agrees with the Dossier Submitter that the assay showed a negative result for chromosomal aberrations.

<u>In vitro gene mutation assay in mammalian cells (L5178Y) (KPIC/BRA, MGK and SCJ, 2001)</u>

This gene mutation test at the TK-locus of L5178Y mouse lymphoma cells with pyrethrin extract (FEK-99 blend; purity: 57.03%) is a GLP compliant study, performed in line with OECD TG 476 with a deviation that was not considered (in the DAR) to affect the

[17] OECD (2016b) Guidance states for *in vitro* cytogenicity assays: "It is now recommended that if the maximum concentration is based on cytotoxicity, the highest concentration should aim to achieve 55±5% cytotoxicity using the recommended cytotoxicity parameters (i.e. reduction in RICC, RPD, CBPI, RI, or MI to 45±5% of the concurrent negative control). Care should be taken in interpreting positive results only found in the higher end of this 55±5% cytotoxicity range." RICC = Relative Increase in Cell Count; RPD = Relative Population Doubling; CBPI = Cytokinesis Blocked Proliferation Index; RI = Replication Index; MI = Mitotic Index

scientific validity and interpretation of the results (the exposure period was 24 hours with and without metabolic activation, whereas the test guideline states that usually three to six hours is effective).

Three assays were carried out:

- Test 1: TK assay with and without metabolic activation at 0.39 - 1200 µg/mL;
- Test 2: TK assay at 3.0 - 50 µg/mL without metabolic activation, and at 3.0 - 100 µg/mL with metabolic activation; and
- Test 3: TK assay with metabolic activation at 13 - 72 µg/mL.

Since in the first assay pyrethrin extract was toxic to cells above 50 µg/mL in the absence of S9-mix, additional cytotoxicity and mutagenicity tests were conducted in a second assay, in which pyrethrum extract was 90% cytotoxic at 85 µg/mL.

Cytotoxicity was determined in each test measuring the relative total growth (RTG).

In the absence of metabolic activation, mutant frequency was not significantly increased at any dose level in either the first or the second assay.

In the presence of S9-mix, the positive control in Test 1 did not comply with the criteria of validation (positive and negative controls had similar TK mutant frequencies). Therefore, this assay was not considered to be valid. In the second assay, the mutant frequency was significantly increased at concentration of 85 and 52 µg/mL of the test substance, and equivocal responses were observed at concentrations of 61 and 26 µg/mL. Nevertheless, no clear dose-response was observed at doses between 26 and 72 µg/mL, and RTG at 85 µg/mL, at which a clear increase was noted (509 vs. 82 – 95 mutant frequency in vehicle control), was only 3% (study results are available in the DAR, Table B.6.68). In the third assay no significant increase of the mutant frequency at any dose level was observed.

RAC agrees with the Dossier Submitter that the test substance was not mutagenic at the TK-locus of mouse lymphoma L5178Y cells.

<u>In vitro gene mutation studies in mammalian cells (L5178Y cells) (KPIC, 2016a; MGK, 2016b; BRA, 2016c)</u>

In these GLP compliant studies performed in line with OECD TG 490 (with no deviations, according to the RAR, 2021), three pyrethrum extract test items were examined for the potential to induce gene mutations at the TK-locus of cultured mouse lymphoma L5178Y cells, both with and without S9 metabolic activation:

- Pyrethrum Extract 50% (purity: 50.24%; correction factor of 1.99 was applied);
- Pyrocide 50% (purity: 53.72%; correction factor of 1.86 was applied); and
- PY-T-50 Pale Refined Pyrethrins (purity: 49.35%; correction factor of 2.03 was applied).

Concentrations applied in the experiments (according to the RAR) were:

- for Pyrethrum Extract 50% (KPIC): 0.025-0.120 µg/mL in the short-term treatment without S9; 0.0005-0.085 µg/mL in the short-term treatment with S9; 0.02-0.085 µg/mL in the long-term treatment without S9;
- for Pyrocide 50% (MGK): 0.010-0.0375 µg/mL in the short-term treatment without S9; 0.015-0.080 µg/mL in the short-term treatment with S9; 0.001-0.020 µg/mL in the long-term treatment without S9;

- for PY-T-50 Pale Refined Pyrethrins (BRA): 0.0025-0.035 µg/mL in the short-term treatment without S9; 0.005-0.080 µg/mL in the short-term treatment with S9; 0.0025-0.024 µg/mL in the long-term treatment without S9.

For all three pyrethrum extracts, tested up to cytotoxic concentrations in the absence and presence of metabolic activation, the Global Evaluation Factor (GEF) of 126 x 10^{-6} was not exceeded at any concentration, and colony sizing showed no clastogenic effects (no numerical data were available to RAC).

RAC concludes that based on the information provided in the CLH report and RAR, the tested extracts did not induce mutations or chromosomal aberrations in these three assays.

<u>In vitro cytogenicity studies in mammalian cells (MN) (KPIC, 2016a; MGK, 2016b; BRA, 2016c)</u>

In these GLP compliant studies performed in line with OECD TG 487 (with no deviations, according to the RAR, 2021), three pyrethrum extract test items were assessed for their potential to induce micronuclei formation in Chinese hamster V79 cells, both with and without S9 metabolic activation:

- Pyrethrum Extract 50% (purity: 50.24%; correction factor of 1.99 was applied);
- Pyrocide 50% (purity: 53.72%; correction factor of 1.86 was applied); and
- PY-T-50 Pale Refined Pyrethrins (purity: 49.35%; correction factor of 2.03 was applied).

Concentrations applied in the experiments (according to the RAR) were:

- for Pyrethrum Extract 50% (KPIC): 0.0010-0.0050 µg/mL in the short-term treatment without S9; 0.0125-0.050 µg/mL in the short-term treatment with S9; 0.0010-0.010 µg/mL in the long-term treatment without S9;
- for Pyrocide 50% (MGK): 0.0025-0.0050 µg/mL in the short-term treatment without S9; 0.025-0.085 µg/mL in the short-term treatment with S9; 0.0025-0.0075 µg/mL in the long-term treatment without S9;
- for PY-T-50 Pale Refined Pyrethrins (BRA): 0.0025-0.0060 µg/mL in the short-term treatment without S9; 0.025-0.10 µg/mL in the short-term treatment with S9; 0.0025-0.010 µg/mL in the long-term treatment without S9.

Adequate cytotoxicity (55% cytotoxicity, according to OECD 2016b) was achieved in a majority of the experiments. Adequate response in positive control was presented as a response to EFSA's request for additional information.

No numerical data on micronuclei formation were available to RAC, but RAC concludes that based on the information provided in the CLH report and RAR, none of the three test items induced micronuclei in Chinese hamster V79 cells *in vitro*, either in absence or presence of metabolic activation.

In vivo studies

Two *in vivo* studies were available to RAC: one old micronucleus test in rodents (KPIC, 1976), which was considered in the DAR and RAR as not acceptable, and one reliable, new study (Micronucleus test in bone marrow cells of the rat with Pyrocide 50%; KPIC, MGK, BRA, 2022), submitted in response to EFSA's request for additional information.

Micronucleus test in rodents (KPIC, 1976)

RAC agrees with the Dossier Submitter that the assay showed negative results. However, this is a non-GLP study. According to the RAR, test guideline was not specified in the report but the method conforms to 92/69/EEC B.12 (corresponds to OECD TG 474). This study was not considered acceptable in the DAR (2007) and RAR (2021) since it does not include a positive control and bone marrow toxicity was not demonstrated (however, maximum tolerated dose was applied, demonstrated by lethality at the highest dose).

RAC considers that the study is not sufficiently reliable to be used for genotoxicity assessment of the test substance, especially since there is a new, reliable *in vivo* micronucleus study available (described below).

Micronucleus test in bone marrow cells of the rat with Pyrocide 50% (KPIC, MGK, BRA, 2022)

This is a GLP compliant study, conducted in line with OECD TG 474. Pyrocide 50% (batch 11831; purity: 52.7%) was administered to rats at a maximum tolerated acute dose. Based on the results of the dose-range finding study test concentrations of 500 mg/kg bw/day for male animals and of 250 mg/kg bw/day for female animals were selected as maximum dose for the main test (5 animals/sex/group).

In the main study animals were dosed twice with a 24-hour interval, by oral gavage with vehicle (corn oil) or with 125, 250 and 500 mg test material per kg body weight for males, or 62.5, 125 and 250 mg test material per kg body weight for females.

Bone marrow was sampled 48 hours after the first dosing and the number of micronucleated polychromatic erythrocytes per 4000 polychromatic erythrocytes was measured in rat bone marrow. A negative (vehicle) and positive control group (cyclophosphamide) showed adequate responses. The incidences of micronucleated polychromatic erythrocytes in the bone marrow of negative and positive control animals were within the 95% control limits of the distribution of the respective historical control database.

Clinical signs of toxicity were limited to the high dose group (tremors) and 2 male animals in the high dose group died. No biologically relevant increases in the mean frequency of micronucleated polychromatic erythrocytes were observed in the bone marrow of male or female animals treated with the test material compared to the vehicle control (study results are presented in "Supplemental information - In depth analyses by RAC", background document).

Males and females treated with the highest test concentration and females treated with lowest test concentration showed a statistically significant decrease in the ratio of polychromatic to normochromatic erythrocytes, demonstrating toxic effects on erythropoiesis.

RAC agrees with the Applicant that the test substance did not show clastogenic or aneugenic effects in the bone marrow micronucleus test of male and female animals up to a dose of 500 or 250 mg/kg bw/day, respectively.

Comparison with the criteria

The genotoxicity of pyrethrum extract has been adequately investigated in battery of

standard tests.

RAC considers that *Chrysanthemum cinerariaefolium* extract did not show genotoxic potential either in *in vitro* or *in vivo* assays. A positive response was observed only for one strain (*S. typhimurium* TA100, with and without metabolic activation) in one bacterial gene mutation assay (KPIC/BRA, MGK and SCJ, 1989). However, this strain was negative in three other bacterial gene mutation assays (KPIC, 2016a; MGK, 2016b; BRA, 2016c). Also, other *S. typhimurium* and *E. coli* strains were negative in all four available bacterial gene mutation assays.

The test substance was found negative in other *in vitro* studies: unscheduled DNA synthesis, mammalian gene mutation and chromosomal aberration tests, and an *in vitro* micronucleus test. Pyrethrum extract was also found negative in a reliable *in vivo* micronucleus test. It is, therefore, concluded that *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent is not genotoxic. This is supported by *in silico* models (Derek Nexus, version 1.1, Lhasa Limited, Leeds, Yorkshire, UK), which predicted no alerts for mutagenicity and chromosome damage *in vivo*.

RAC supports the Dossier Submitter's proposal **for no classification for germ cell mutagenicity**.

Supplemental information - In depth analyses by RAC

Results from the 1st bacterial gene mutation assay for *S. typhimurium* strain TA100 (KPIC; BRA, MGK and SCJ, 1989):

	Average revertants per plate ± SD for TA100			
Dose (µg)	**-S9**	**-S9 conf.**	**+S9**	**+S9 conf.**
0*	109 ± 6	94 ± 6	136 ± 12	115 ± 14
292	99 ± 7	87 ± 10	133 ± 8	122 ± 9
585	137 ± 24	94 ± 1	141 ± 20	136 ± 4
877	124 ± 16	98 ± 3	133 ± 6	123 ± 6
2924	125 ± 6	118 ± 18	146 ± 10	155 ± 7
5848	148 ± 12	127 ± 8	150 ± 8	167 ± 9
8772	152 ± 9	157 ± 10	155 ± 5	182 ± 20
Pos**	394 ± 50	337 ± 60	523 ± 18	731 ± 53

*Vehicle control; **Positive control – sodium azide 1.0 µg per plate; conf. – confirmatory assay

Type of chromosomal aberrations and cytotoxicity in *in vitro* mammalian chromosomal aberration test in CHO-K1 cells (KPIC/BRA, MGK and SCJ, 1996):

		Chromatid aberration					**Chromatid aberation**		
Dose	**MI (%)**	**Gap**	**Break**	**Exch**	**Dose**	**MI (%)**	**Gap**	**Break**	**Exch**
12-h harvest, -S9					**12-h harvest, +S9**				

DMSO	6.4 (100)	0.5	0.5	0	DMSO	4.7 (100)	0.5	1.5	0
TM 40	5.1 (80)	0.5	0	0	TM 25	7 (149)	1.5	2	0
TM 55	4.1 (64)	0.5	0.5	0	TM 40	6 (128)	1	1	0
TM 70	3.8 (59)	1	1.5	0	TM 55	3.6 (77)	1.5	2.5	0
TM 85	1.2 (19)	0	0.5	0	TM 70	1.4 (30)	2.5	0.5	0
24-h harvest, -S9					**24-h harvest, +S9**				
DMSO	6.7 (100)	0	0	0	DMSO	9.5 (100)	1	0	1
TM 12.5	5.6 (84)	1	0	0	TM 12.5	9.6 (191)	1	0.5	1.5
TM 25	9 (134)	0	2	0	TM 25	12 (126)	0.5	0.5	1
TM 50	3.1 (46)	0.5	0	0	TM 50	6.6 (69)	0.5	1.5	0.5
TM 100	2.1 (31)	2.5	1.5	0	TM 100	1.9 (20)	1.5	2.5	0.5
48-h harvest, -S9					**48-h harvest, +S9**				
DMSO	7.4 (100)	0	0.5	0	DMSO	11.2 (100)	0	1.5	0
TM 6.25	7.6 (103)	0.5	0	0	TM 40	9.6 (86)	1	0	0
TM 12.5	7.6 (103)	0.5	0.5	0	TM 55	6.7 (60)	0	1.5	0
TM 25	6.3 (85)	0.5	0	0.5	TM 70	8.7 (78)	1	0	0.5
TM 50	1.3 (18)	1	1.5	0	TM 85	4.8 (43)	3	1.5	4.5

Data are expressed as average for two analysed flasks per dose level. Dose (treatment) is expressed in µg/mL; MI - mitotic index, also expressed as percent of DMSO (control) (in brackets); Exch – chromatid exchange

Mean number of micronucleated polychromatic erythrocytes and ratio of polychromatic/normochromatic erythrocytes in *in vivo* micronucleus test in bone marrow cells of the rat with Pyrocide 50% (KPIC, MGK, BRA, 2022):

Treatment	**Dose** (mg/kg bw)	**Number of micronucleated polychromatic erythrocytes** (mean±SD)	**Ratio polychromatic /normochromatic erythrocytes** (mean±SD)
MALES			
Vehicle control	0	4.6 ± 2.3	1.92 ± 0.27
PYROCIDE 50%	125	7.2 ± 3.1	2.21 ± 0.07
PYROCIDE 50%	250	6.6 ± 2.3	1.61 ± 0.32
PYROCIDE 50%	500	3.0 ± 0	1.37* ± 0.08
CP	19	15.3* ± 1.5	0.88* ± 0.10
FEMALES			
Vehicle control	0	2.6 ± 1.7	2.19 ± 0.23

PYROCIDE 50%	62.5	3.4 ± 1.8	1.35* ± 0.11
PYROCIDE 50%	125	3.0 ± 1.0	1.96 ± 0.37
PYROCIDE 50%	250	3.0 ± 1.7	1.55* ± 0.31
CP	19	19.7* ± 2.5	0.55* ± 0.15

There were five animals per treatment group in control (corn oil) and PYROCIDE exposed groups, and three animals per group in positive control group; CP – cyclophosphamide; *Significantly different from corresponding control group

In silico models on mutagenicity

In response to EFSA's request for additional information on *in silico* predictions for photomutagenicity of pyrethrins, the Applicant presented *in silico* models using Derek Nexus (version 1.1, Lhasa Limited, Leeds, Yorkshire, UK; Knowledge Base: Derek KB 2018 1.1). Derek Nexus predicted that pyrethrins do not have alerts regarding photomutagenicity. There were alerts for mutagenicity for certain pyrethrin components (e.g. chromosome damage *in vitro* in mammal cells predicted as plausible for pyrethric acid and equivocal for pyrethrin 1, pyrethrolone, dihydroxy-pyrethrolone-1, dihydroxy-pyrethrolone-2, cinerolone, jasmoline). However, all substances submitted for *in silico* assessment were predicted to be inactive in the bacterial *in vitro* (Ames) mutagenicity test and there were no alerts either for mutagenicity or chromosome damage *in vivo*.

A.2.9. Carcinogenicity

In a 2-year dietary chronic toxicity and oncogenicity study on rats Pyrethrum extract corresponding to 3000 ppm total pyrethrins (199/265 mg/kg bw/d extract males/females) caused effects in both sexes: body weight gains were occasionally reduced and an increased incidence of hyperplasia and follicular cell adenomas at the thyroid was observed. In addition, males showed increased and accentuated lobulations of the liver, increased activity of serum transaminases and increased numbers of keratoacanthomas, while in females the incidence of hepatic adenoma was increased slightly.

At 1000 ppm total pyrethrins (66/86 mg/kg bw/d extract males/females) led to an accentuated lobulation of the liver and an increase in the incidence of hyperplasia and follicular cell adenomas of the thyroid, all in males only.

The NOAEL was 100 ppm actual Pyrethrins, equal to 4 mg/kg bw/d total pyrethrins (6 mg/kg bw/d extract) in males and 5 mg/kg bw/d total pyrethrins (8 mg/kg bw/d extract) in females ([redacted]). (KPIC) (BRA, MGK and SCJ)

		Males					Females				
		Control 1	Control 2	100 ppm	1000 ppm	3000 ppm	Control 1	Control 2	100 ppm	1000 ppm	3000 ppm
Body weight (g)	**Mean**	646	616	592	607	589	419	506*	478	439	408
	SD	108.5	112.9	103.4	122.7	107.5	94.1	120.6	107.2	116.3	84.8
	N	32	27	22	38	33	26	15	20	28	32
Brain (g)	**Mean**	2.26	2.23	2.25	2.26	589	2.06	2.01	2.07	2.05	2.05
	SD	0.159	0.132	0.145	0.138	107.5	0.079	0.121	0.133	0.141	0.110
	N	33	27	22	38	33	27	15	20	28	32
Brain/Body weight (%x10)	**Mean**	3.58	3.72	3.94	3.80	3.97	5.19	4.21	4.54	4.95	5.27**
	SD	0.575	0.600	0.825	0.738	0.856	1.343	1.136	1.104	1.158	1.234
	N	32	27	22	37	33	26	15	20	28	32
Liver weight (g)	**Mean**	24.31	22.01	24.72	24.353	24.86	16.99	19.69	18.25	17.75	18.69
	SD	4.697	4.490	5.450	4.463	5.534	3.005	4.155	4.582	4.881	3.485
	N	33	26	21	39	33	26	15	20	28	32
Liver/Body weight (%)	**Mean**	3.83	3.64	4.25**	4.08	4.25**	4.18	3.99	3.86	4.09	4.67**
	SD	0.751	0.709	1.065	0.854	0.718	1.056	0.746	0.668	0.768	0.817
	N	32	26	21	38	33	25	15	20	28	32
Liver/Brain weight (%x10^{-2})	**Mean**	10.79	9.93	11.03	10.90	11.03	8.25	9.76	8.87	8.65	9.12
	SD	2.115	2.039	2.591	1.961	2.481	1.405	1.880	2.371	2.363	1.760
	N	33	26	21	38	33	26	15	20	28	32

*Significantly different from Control group 1 ($p < 0.05$)

**Significantly different from Control group 2 ($p < 0.05$)

		Males										Females									
		Control 1		Control 2		100 ppm		1000 ppm		3000 ppm		Control 1		Control 2		100 ppm		1000 ppm		3000 ppm	
		DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS
N (examined)		27	33	33	27	38	22	21	39	27	33	33	27	45	15	40	20	32	28	28	32
LIVER																					
Accentuated lobulation	**Mild**	3.7	12.1	6.1	11.1	5.3	31.8		30.8	11.1	27.3		14.8	6.7	13.3		5.0	12.5	14.3	3.6	9.4
	Moderate	3.7		6.1		5.3		19.1		7.4		3.0		2.2		2.5		3.1		7.1	
	Severe			3.0				4.8								2.5					
Adhesions	**Mild**					5.3															
Congested	**Mild**																			3.6	
Cyst, clear/NOS	**Mild**						4.6			3.7											3.1
	Moderate												3.7		6.7						
Discolored, tan	**Mild**		21.2		7.4	5.3	4.6	4.8	2.6	3.7	9.1		3.7	6.7	13.3	5.0			3.6		3.1
	Moderate	3.7		3.0			4.6													3.6	
	Severe			6.1					2.6									3.1			
Enlarged	**Mild**	3.7																		3.6	
	Moderate																	3.1			
	Severe											3.0									
Foci/focus, red/dark	**No grade**													2.2							
	Trace			3.0				4.8						2.2							

red/black/dark	**Mild**	18.5	36.4	27.3	44.4	29.0	45.5	14.3	43.6	22.2	36.4	15.2	44.4	22.2	53.3	15.0	55.0	15.6	60.7	25.00	50.0
	Moderate	18.5		6.1				9.5	2.6					6.7		2.5		6.3		10.7	
Foci/focus, tan/yellow/ white/NOS	**No grade**					2.6		4.8													
	Trace			3.0																	
	Mild	18.5	18.2	6.1	11.1	10.5	13.6	23.8	7.7	11.1	18.2	18.2	18.5	2.2	26.7	5.0	5.0	9.4	3.6	10.7	21.9
	Moderate			3.0				4.8				6.1				5.0		3.1			
	Severe													2.2				3.1			
Foci, gray	**Mild**	3.7																			
Foci, clear	**Mild**				3.7						3.0										
Firm	**Mild**				3.7											2.5					
Friable/rupture	**Mild**								2.6		6.1										
	Moderate			6.1			4.6										5.0	3.1		3.6	
Granular	**Mild**			3.0			4.6														
	Moderate																	3.1			
Mass		7.4	3.0			2.6				3.7	3.0		3.7	2.2				3.1	7.1	3.6	3.1
Mottled	**Mild**	7.4		3.0		10.5						3.0		4.4		7.5		3.1			
	Moderate			6.1		2.6				3.7		12.1				5.0		3.1			
	Severe	3.7				5.3								2.2		2.5					
Nodule									2.6			3.0								3.6	
Pale	**Mild**	11.1				2.6															
	Moderate					2.6				3.7						2.5				3.6	
	Severe					5.3															
Tan	**Moderate**			6.1																	
THYROID																					
Discolored, tan	**Mild**						4.6														
Enlarged	**Trace**			3.0																	
	Mild				7.4		4.6				3.0		3.7						3.6		9.4
	Moderate							4.8	2.6	3.7			3.7	2.2							

DOS – Deaths and unscheduled sacrifices
TS – Terminal sacrifice
NOS – Not otherwise specified

		Males										**Females**									
		Control 1		**Control 2**		**100 ppm**		**1000 ppm**		**3000 ppm**		**Control 1**		**Control 2**		**100 ppm**		**1000 ppm**		**3000 ppm**	
		DOS	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**
N (examined)		27	33	33	27	38	22	21	39	27	33	33	27	45	15	40	20	32	28	28	32
LIVER																					
Within normal limits		14.8	21.2	12.1	22.2	18.4	13.6	9.5	7.7	29.6	12.1	48.5	22.2	44.4	20.0	50.0	45.0	31.3	21.4	32.1	9.4
Abscess	**Severe**															2.5					
Autolysis too severe for diagnosis														2.2							
Bile duct hyperplasia	**Trace**	18.5	36.4	21.2	18.5	26.3	4.6	9.5	48.7	7.4	33.3	12.1	14.8	4.4	6.7	7.5	15.0	15.6	25.0	17.9	28.1
	Mild	22.2	15.2	21.2	33.3	13.2	50	23.8	20.5	3.7	27.3		14.8	8.9		7.5	5.0	12.5	28.6		21.9
	Moderate			3.0	11.1	2.6				7.4			3.7			2.5					3.1
Cholangioma													3.7		6.7						3.1
Cholangiofibrosis	**Trace**									3.7											

	Mild	3.7									3.0									
	Moderate	3.7	3.0																	
Cyst	**Mild**						4.6	4.8												
Cytoplasmic alteration, basophilic	**Trace**		12.1		11.1	2.6			2.6	3.7	6.1		14.8		13.3	2.5	15.0	3.1	7.1		9.4
	Mild		9.1	6.1	11.1	5.3	4.6		10.3	3.7	3.0	6.1	25.9	8.9	46.7	2.5	15.0		10.7		15.6
	Moderate		3.0		7.4			4.8	7.7	3.7	3.0	3.0		2.2	13.3	2.5	5.0		7.1	3.6	9.4
Cytoplasmic alteration, clear	**Trace**		12.1		18.5		9.1		2.6		6.1		3.7		20.0						3.1
	Mild		6.1		3.7				10.3	3.7	21.2		3.7		6.7				10.7		6.3
	Moderate				3.7		4.6					3.0	3.7						3.6		
Cytoplasmic alteration, eosinophilic	**Mild**										6.1						5.0				
	Moderate								5.1												
Hematopoiesis, extramedullary	**Trace**				3.7							3.0		2.2		7.5		3.1		10.7	
	Mild							4.8				3.0									
	Moderate												3.7	4.4							
Fibrosis	**Trace**									3.7			7.4								
	Mild					2.6								2.2		2.5					
Hepatocellular adenoma		11.1	9.1	3.0				9.5	2.6		9.1			2.2					3.6	7.1	9.4
Hepatocellular carcinoma		3.7								3.7			3.7								
Hemorrhage	**Trace**						4.6														
	Mild					2.6	4.6														
Hemolymphoreticular neoplasm		7.4		3.0		7.9		4.8	2.6	11.1		3.0		2.2		5.0		6.3		7.1	
Inflammation	**Trace**	3.7	9.1	3.0		2.6	4.6	4.8	2.6	7.4					6.7	2.5		3.1			6.3
	Mild	3.7	6.1	6.1	3.7	5.3					3.0							3.1		3.6	
	Moderate									3.7											
Metastatic tumor																		3.1		3.6	
Spongiosis hepatis	**Trace**	3.7	21.2	9.1	14.8	5.3	27.3	9.5	12.8	11.1	30.3			2.2					7.1		
	Mild		6.1		11.1				7.7		18.1									3.6	
	Moderate		3.0						2.6	7.4											
Necrosis	**Trace**	3.7	3.0	6.1					7.7			3.0		2.2					3.6	3.6	3.1
	Mild			3.0		13.2		14.3		3.7		3.0		4.4	6.7	10.0		3.1		3.6	
	Moderate	3.7		3.0										2.2						10.7	
	Severe	3.7		3.0		7.9										2.5		3.1		3.6	
Pigment, brown	**Trace**			3.0		2.6									6.7						
	Moderate												3.7								
Telangiectasis	**Trace**	11.1																			3.1
	Mild	3.7	6.1						2.6			3.0		4.4					3.6		6.3
	Moderate					2.6			2.6			3.0				5.0		3.1	3.6		
Vacuolar change	**Trace**				3.7		4.6														
Vacuolar change, fatty	**Trace**		3.0	3.0		10.5		14.3	2.6			3.0	7.4	8.9		2.5		9.4		3.6	
	Mild	22.2		15.2		15.8	4.6	9.5	2.6	11.1		12.1	3.7	8.9		2.5		9.4		3.6	
	Moderate			9.1	3.7	2.6		4.8		7.4				2.2		5.0					
	Severe							4.8					7.4	2.2				3.1			
Cytoplasmic	**Mild**												3.7				10.0				

alteration, vacuolated	**Moderate**											3.0	3.7							3.1
	Severe															2.5				
THYROID																				
Within normal limits		44.4	51.5	48.5	33.3	68.4	68.2	76.2	53.9	55.6	54.6	72.7	44.4	60.0	53.3	77.5	40.0	56.3	89.3	60.7	46.9
Cyst, colloid	**Trace**		3.0																		
	Mild			6.1							3.0			2.2							
	Moderate																				3.1
Cyst	**Mild**																				3.1
Corpora amylacea				3.0																	
Follicular adenoma		3.7	3.0			7.9		9.5	7.7	11.1	6.1						10.0	9.4		3.6	12.5
Follicular carcinoma					3.7	2.6			5.1		6.1		3.7	4.4							3.1
Hyperplasia	**Trace**						4.6		5.1								5.0				
	Mild	3.7	3.0						7.7	11.1	12.1			2.2				3.1		3.6	12.5
	Moderate					2.6								2.2							
Inflammation	**Trace**			3.0																3.6	
Mineralization	**Trace**	3.7	9.1	3.0	3.7		4.6				9.1										3.1
	Mild										3.0					2.5					
Parafollicular cell adenoma		11.1	9.1	9.1	11.1	10.5	4.6	4.8	5.1		12.1		11.1	4.4		2.5	20.0	9.4	3.6	3.6	15.7
Parafollicular cell carcinoma		3.7	3.0		7.4				5.1				7.4			2.5					
Parafollicular cell hyperplasia	**Trace**	7.4		3.0					2.6			3.0	3.7	4.4	6.7	5.0	15.0	9.4		7.1	3.1
	Mild	11.1	6.1		14.8		4.6		2.6	3.7	3.0	6.1	7.4	2.2	6.7	2.5	5.0		3.6		9.4
	Moderate		3.0																		
Ultimobranchial cyst		22.2	27.3	33.3	33.3	10.5	13.6	9.5	12.8	22.2	12.1	24.2	29.6	28.9	33.3	7.5	10.0	18.8	7.1	21.4	6.3

DOS – Deaths and unscheduled sacrifices
SAC – Terminal sacrifice

In an 18-month dietary oncogenicity study on mice, from 2500 ppm total pyrethrins (530/633 mg/kg bw/d extract) onwards discoloured dark livers and increased absolute and relative liver weights were observed in males and females, and vacuolar fatty changes in the livers of males only. Lung carcinomas in males were not treatment related. For females, there was no evidence for carcinogenic response.

The NOEL/NOAEL was 100 ppm actual Pyrethrins, equal to 14/17 mg/kg bw/d total pyrethrins (22/26 mg/kg bw/d extract) for males/females [redacted]. (KPIC and BRA, MGK and SCJ)

		Males					Females				
		Control 1	Control 2	100 ppm	2500 ppm	5000 ppm	Control 1	Control 2	100 ppm	2500 ppm	5000 ppm
Body weight (g)	**Mean**	40	40	40	40	40	35	35	35	36	34
	SD	3.5	3.3	3.5	3.0	3.2	3.6	3.4	3.7	3.8	3.0
	N	42	48	44	40	44	45	41	49	44	42
Brain (g)	**Mean**	0.53	0.53	0.53	0.54	0.54	0.54	0.55	0.55	0.55	0.54
	SD	0.031	0.029	0.029	0.035	0.030	0.027	0.038	0.038	0.031	0.026
	N	42	48	44	40	44	45	41	49	44	42
Brain/Body weight (%x10)	**Mean**	13.3	13.4	13.2	13.7	13.6	15.9	15.9	15.8	15.6	15.8
	SD	1.20	1.27	1.13	1.02	1.13	1.79	1.93	1.74	1.70	1.41
	N	42	48	44	40	44	45	41	49	44	42
Liver weight (g)	**Mean**	2.29	2.33	2.38	2.89*,**	3.10*,**	2.17	2.18	1.99***	2.68**,***	2.90*,**
	SD	0.352	0.333	0.398	0.347	0.412	1.016	0.340	0.409	0.792	0.464
	N	39	44	44	40	38	45	41	49	44	42
Liver/Body weight (%)	**Mean**	5.77	5.81	5.92	7.30*,**	7.91*,**	6.20	6.29	5.68**	7.48*,**	8.46*,**
	SD	0.775	0.717	1.010	0.712	0.751	2.197	0.710	1.042	1.662	0.987
	N	39	44	44	40	38	45	41	49	44	42
Liver/Brain weight (%x10^{-2})	**Mean**	4.33	4.38	4.50	5.37*,**	5.79*,**	4.01	4.00	3.65****	4.88**,***	5.40*,**
	SD	0.683	0.678	0.753	0.654	0.673	1.862	0.678	0.761	1.482	0.849
	N	39	44	44	40	38	45	41	49	44	42

*Significantly different from Control group 1 ($p < 0.01$)
**Significantly different from Control group 2 ($p < 0.01$)
***Significantly different from Control group 1 ($p < 0.05$)
****Significantly different from Control group 2 ($p < 0.05$)

		Males										Females									
		Control 1		Control 2		100 ppm		2500 ppm		5000 ppm		Control 1		Control 2		100 ppm		2500 ppm		5000 ppm	
		DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS
N (examined)		18	42	12	48	16	44	20	40	16	44	15	45	19	41	11	49	16	44	18	42
LIVER																					
Accentuated lobulation	**Mild**					6.3	2.3	5.0	7.5		9.1									5.6	2.4
Adhesions	**Mild**		2.4																		
	Moderate													5.3							
Cyst, clear	**Mild**																		4.6		
Autolysis	**Severe**	5.6																			
Discolored,	**Mild**				2.1				2.5	6.3	43.2								15.9	5.6	31.0

dark/brown	**Moderate**																		5.6		
Discolored, tan	**Moderate**	5.6				6.3													5.6		
	Severe					6.3						6.7									
Enlarged	**Mild**					6.3								10.5					2.3		
	Moderate			8.3						6.3			2.2						2.3		
Foci/focus, red/black/dark	**Mild**	5.6					4.6					6.7							2.3		2.4
	Moderate							5.0													
Foci/focus, tan/white	**Trace**																	6.3			
	Mild	5.6	2.4			18.8	2.3	5.0		12.5	6.8		4.4			9.1				5.6	2.4
	Moderate													5.3							
	Severe																	6.3			
Friable/rupture	**Moderate**							5.0												5.6	
Granular	**Mild**												2.2		2.4				2.3		
	Moderate																		2.3		
Mass		5.6	14.3	8.3	20.8	6.3	6.8	20.0	20.0	6.3	20.5			5.3	2.4	9.1		6.3			2.4
Mottled	**Moderate**			8.3																	
Nodule		5.6	2.4	8.3	6.3		2.3		2.5		4.6		2.2			9.1	2.0				
Pale	**Severe**															9.1					
THYROID																					
Enlarged	**Mild**														2.4			6.3			
	Moderate																				2.4

DOS – Deaths and unscheduled sacrifices
TS – Terminal sacrifice

		Males										**Females**									
		Control 1		**Control 2**		**100 ppm**		**2500 ppm**		**5000 ppm**		**Control 1**		**Control 2**		**100 ppm**		**2500 ppm**		**5000 ppm**	
		DOS	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**
N (examined)		18	42	12	48	16*	44*	20*	40*	16	44	15	45	19	41	11*	49*	16**	44*	18	42
LIVER																					
Within normal limits		50.0	50.0	75.0	45.8	31.3	40.9	40.0	22.5	37.5	34.1	53.3	35.6	47.4	24.4	36.4	40.8	37.5	36.4	27.8	47.6
Abscess	**Severe**							5.0													
Amyloidosis	**Trace**	5.6	2.4		8.3		4.6		2.5		2.3		8.9	10.5	9.8		6.1				
	Mild	5.6	2.4		4.2	25.0		5.0	7.5	25.0	2.3	20.0		10.5	4.9	9.1		18.8		11.1	
	Moderate							20.0		12.5						9.1		25.0		33.3	2.4
Autolysis too severe for diagnosis						6.3										9.1					
Cystic dilatation	**Mild**																		2.3		
	Moderate																		2.3		
	Severe																		2.3		
Cyst	**Mild**				2.1																
Cytoplasmic alteration, basophilic	**Trace**				2.1										2.4						
	Mild								5.0		2.3		2.2	5.3							
	Moderate								2.5			6.7			2.4						
Cytoplasmic	**Trace**				2.1																

alteration, clear																				
Cytoplasmic alteration, eosinophilic	**Moderate**				2.1				2.5								2.0		2.3		
Hematopoiesis, extramedullary	**Trace**											6.7			2.4	9.1				5.6	4.8
	Mild	11.1		8.3								6.7									
	Moderate					6.3						6.7		5.3		9.1					
Fibrosis	**Mild**													5.3							
	Moderate									6.3											
Hepatocellular adenoma		5.6	9.5	8.3	22.9	6.3	6.8	10.0	22.5		22.7		2.2							5.6	
Hemangiosarcoma					4.2		4.6	5.0	2.5		2.3							6.3			2.4
Hepatocellular carcinoma		5.6	7.1	8.3	4.2	6.3				6.3	4.6										2.4
Hemorrhage	**Mild**							5.0				6.7									
Hemolymphoreticular neoplasm		5.6											4.4	10.5		9.1	2.0	12.5	6.8		4.8
Inflammation	**Trace**		14.3		27.1		31.8	5.0	22.5		18.2	6.7	33.3	10.5	46.3		32.7		36.4		26.2
	Mild	5.6	27.8		2.1	6.3	11.4		5.0		2.3		13.3	10.5	14.6		14.3	6.3	4.6		2.4
	Moderate		2.4										6.7	5.3							
	Severe									6.3											
Infarct	**Severe**													5.3							
Lymphocitic infiltration	**Trace**		2.4				9.1	5.0	7.5	6.3	2.3				9.8	9.1	2.0	6.3	9.1	5.6	7.1
	Mild						2.3						2.2				4.1				
Plasma cell infiltration	**Mild**												2.2								
Mineralization	**Trace**				2.1															5.6	
Metastatic tumor		5.6						5.0													
Necrosis	**Trace**	5.6	2.4		4.2					6.3							12.3		2.3		
	Mild		2.4			6.3	4.6							5.3	2.4			6.3	2.3	5.6	
	Moderate				2.1																
Pigment, brown	**Trace**		2.4		2.1		4.6		5.0		4.6	13.3	6.7	15.8	17.1		10.2		11.4		11.9
	Mild		4.8								2.3	6.7	6.7					6.3	2.3		9.5
	Moderate									6.3	2.3		2.2							5.6	2.4
Vacuolar change, fatty	**Trace**		2.4			6.3			10.0	6.3	13.6										
	Mild				2.1	6.3			7.5		13.6										
	Moderate								2.5		2.3										
Cytoplasmic alteration, vacuolated	**Mild**				2.1																
THYROID																					
Within normal limits		77.8	76.2	66.7	70.8	-	-	-	-	50.0	86.4	66.7	73.3	79.0	78.1	-	-		-	50.0	78.6
Cyst, colloid	**Trace**	11.1	7.1		12.5	-	-	-	-		4.6		6.7			-	-		-		2.4
	Mild		2.4		4.2	-	-	-	-		4.6	6.7	2.2			-	-		-		2.4
	Moderate					-	-	-	-				2.2			-	-		-		
Amyloidosis	**Trace**		2.4	8.3	4.2	-	-	-	-	12.5		6.7	2.2	5.3	7.3	-	-		-		2.4

	Mild	11.1	9.5	8.3	6.3	-	-	-	-			6.7	11.1	10.5	2.4	-	-		-	5.6	2.4
	Moderate			8.3	4.2	-	-	-	-	12.5	4.6	6.7	2.2		9.8	-	-	6.3	-	27.8	
	Severe		2.4	8.3		-	-	-	-	18.8		6.7		5.3		-	-		-	11.1	2.4
Hemolymphoreticular neoplasm						-	-	-	-					5.3		-	-		-		
Hyperplasia	**Mild**					-	-	-	-				2.2		2.4	-	-		-		2.4
Inflammation	**Trace**					-	-	-	-							-	-		-		4.8
	Mild					-	-	-	-							-	-		-		2.4
	Moderate					-	-	-	-	6.3						-	-		-		
Mineralization	**Trace**					-	-	-	-							-	-		-	5.6	

DOS – Deaths and unscheduled sacrifices
SAC – Terminal sacrifice
*For thyroid, no animals were examined.
**For thyroid, only one animal was examined.

Table A.48 Summary table of carcinogenicity studies in animals

Summary table of carcinogenicity studies in animals						
Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects, for all dose levels)	Remarks (e.g. major deviations)	Reference

Oral 2-year Diet EPA 83-5, OECD 453 GLP Reliability 1 Key	Rat Charles River CD male+female 60/sex/dose	Pyrethrum extract (Blend FEK-99; Purity 57.574%) 0 ppm 100 ppm 1000 ppm 3000 ppm in diet daily *ad libitum*; equivalent to 0, 4, 43, and 130 mg/kg bw/d total pyrethrins (0, 6, 66, and 199 mg/kg bw/d extract) for males and 0, 5, 56 and 173 mg/kg bw/d total pyrethrins (0, 8, 86, and 265 mg/kg bw/d extract) for females	NOAEL: 100 ppm Males: 4 mg/kg bw/d total pyrethrins (6 mg/kg bw/d extract) Females: 5 mg/kg bw/d total pyrethrins (8 mg/kg bw/d extract) LOAEL: 1000 ppm Males: 43 mg/kg bw/d total pyrethrins (66 mg/kg bw/d extract) Females: 56 mg/kg bw/d total pyrethrins (86 mg/kg bw/d extract)	3000 ppm (199/265 mg/kg bw/d extract): hepatotoxicity, thyroidal tumours, liver adenoma (females) body weight ↓ 1000 ppm (66/86 mg/kg bw/d extract): hepatotoxicity (males)	-	[redacted] (KPIC) IIIA6.7/01 [redacted] (BRA, MGK and SCJ) IIIA6.7/1
Oral 18-months diet EPA 83-2, OECD 451 GLP Reliability 1 Key	Mouse Charles River CD male+female 60/sex/dose	Pyrethrum extract (Blend FEK-99; Purity 57.574%) 0 ppm 100 ppm 2500 ppm 5000 ppm in diet daily *ad libitum*;	NOAEL: 100 ppm males: 14 mg/kg bw/d total pyrethrins (22 mg/kg bw/d extract) Females: 17 mg/kg bw/d total pyrethrins	5000 ppm (1051/1278 mg/kg bw/d extract): hepatotoxicity 2500 ppm (530/633 mg/kg bw/d extract): hepatotoxicity	-	[redacted] (KPIC) IIIA6.7/02 [redacted] (BRA, MGK and SCJ) IIIA6.7/2

		equivalent to 0, 14, 346, and 686 mg/kg bw/d total pyrethrins (0, 22, 530, and 1051 mg/kg bw/d extract) for males and 0, 17, 413, and 834 mg/kg bw/d total pyrethrins (0, 26, 633, and 1278 mg/kg bw/d extract) for females	(26 mg/kg bw/d extract) LOAEL: 2500 ppm Males: 346 mg/kg bw/d total pyrethrins (530 mg/kg bw/d extract) Females: 413 mg/kg bw/d total pyrethrins (633 mg/kg bw/d extract)			

Table A.49 Summary table of human carcinogenicity data
No data are available.

Table A.50 Summary table of other relevant studies for carcinogenicity
No data are available.

A2.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Rat

Groups of 60 Charles River CD rats of each sex received diets containing 0, 100, 1000, or 3000 ppm actual Pyrethrins for 104 weeks, equal to 0, 4, 43, and 130 mg/kg bw/d total pyrethrins (0, 6, 66, and 199 mg/kg bw/d extract) for males and 0, 5, 56, and 170 g/kg bw/d total pyrethrins (0, 8, 86, and 265 mg/kg bw/d extract) for females.

No mortalities and no clinical signs attributable to the administration of the test substance were found. Body weights were significantly depressed in both sexes in the 3000 ppm (199/265 mg/kg bw/d extract) treatment group during the first 78 weeks only, combined with a slight decrease in food consumption.

No test substance related organ weight changes and no haematological of urological changes were found. Statistically significant increases in the activity of serum transaminases were determined at most intervals of analysis at males of the 3000 ppm (199 mg/kg bw/d extract) dosage group.

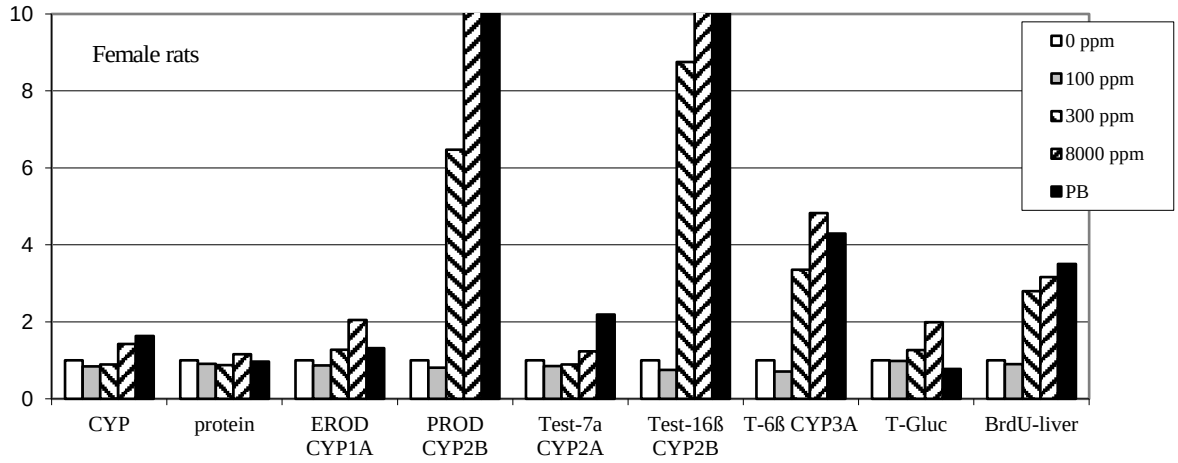
Keratoacanthomas of the skin were increased at the highest dosage level in the male rats, however, due to the self-limiting nature of this lesion, it is unlikely that this finding has any true toxicological significance. Increased incidences of benign tumours of the thyroid were also observed and a statistically significantly higher incidence of hepatocellular adenomas was described in females at the high dose. Follicular adenomas and carcinomas were initially seen in the thyroid glands of rats at the high dose, which appeared to be related to treatment, but during a re-evaluation some of the carcinomas were reclassified as adenomas and some adenomas were reclassified as hyperplasia. After the re-evaluation, the incidence of hyperplasia was found to be enhanced in males and females, and the incidence of follicular adenomas was statistically significantly increased only in females at 3000 ppm (265 mg/kg bw/d extract). The NOEL/NOAEL was 100 ppm, equal to 4 mg/kg bw/d total pyrethrins (6 mg/kg bw/d extract) in males and 5 mg/kg bw/d total pyrethrins (8 mg/kg bw/d extract) in females.

To advance the understanding of the mechanisms by which Pyrethrins cause rodent tumours and to better understand the relevance of the observations in rats to humans, a mechanistic toxicity study in rats was carried out ([redacted] (IIIA6.10/1); [redacted] (IIIA6.10/2)). The aim of both studies was to determine if the *Chrysanthemum cinerariaefolium* extract from HCS is a genotoxic carcinogen or the increase in the incidence of tumours follows a mechanism similar to that for phenobarbital as a consequence of microsomal enzyme induction. The first is focused on carcinogenic effects and the second on enzyme induction in the liver. The test substance (FEK-99) was the same as in the carcinogenicity study in rats (IIIA6.7/1) in the first study. The second study was carried out using liver samples from the previous study taken at different times. The following conclusions can be drawn:

- There is a consensus that pyrethrins are not genotoxic.
- Only benign tumours (adenoma) were observed – no carcinoma – in the 2-year oral toxicity bioassay in rats.
- There was no clear dose response, e.g. as a singularly phenomenon only in females maintained for two years on the (maximum tolerated) high dose (3000 ppm) a minimal increase in adenoma was noted.

- In male rats the incidences of adenoma were 5% (3 of 60 males) in both, the highest dose and the mid dose (3000 ppm and 1000 ppm (199 and 66 mg/kg bw/d extract)).
- Subchronic studies indicated that Pyrethrins induced signs of hepatotoxicity in male and female rats, this was confined to high dose levels (≥ 3000ppm (199/265 mg/kg bw/d extract)).

Figure 2: Graphical presentation of the data obtained in the mechanistic study (14 days of treatment). Data are normalised for values of control animals. A clear parallelism is obvious for high dose Pyrethrins (hatched bars) and Phenobarbital (black bars) while low dose Pyrethrins (grey bars) parallel the untreated controls (white bars).



The mechanistic study was conducted with different concentrations of pyrethrins investigating several time points (7 d, 14 d, 42 d and 42 d treatment + 42 days recovery) including a negative control group and a phenobarbital-treated positive control group.

The parallelism of effects observed in the positive control (~1500 ppm phenobarbital) and the high dose Pyrethrins-treated groups is evident (Figure 2) and the conclusion may be drawn that both compounds induce tumours in the liver by a similar non-genotoxic mechanism at high dose levels. Notably, phenobarbital appears to be far more potent as compared to Pyrethrins, and indeed the effect of pyrethrins on the incidence of hepatic adenoma was only minimal.

Phenobarbital has been shown to induce liver enzymes and also hepatic cell proliferation via an activation of the constitutive androstane receptor (CAR), a nuclear receptor that forms heterodimers with the retinoid acid receptor to induce a number of proteins but not hepatic cytotoxicity. Similar mechanisms have been reported for synthetical analogs of Pyrethrins. Epidemiological studies showed no human cancer risk for Phenobarbital a widely used barbiturate and antiepileptic drug that is taken at considerable (pharmacologic) dose levels, therefore it is highly unlikely that exposure to Pyrethrins at levels below the ADI (0.04 mg/kg bw/d) will have an influence of human cancer risk.

Increased liver weights were observed at high dose levels in both male and female rats in the 2-year chronic bioassay and hepatotoxicity was also observed both in male and female rats in a subchronic study (90 days oral administration [redacted]). Increased liver weights were noted at the high dose level for male rats and for female rats both at 1000 ppm and 3000 ppm total pyrethrins (66/86 and 199/265 mg/kg bw/d extract) [redacted].

This is in concordance with the view, that hepatotoxicity was the mechanism in a 2-year feeding study with rats, where a marginal increase of benign tumours was noted in the liver of females.

It is a general consensus in the scientific community that benign liver tumours induced in the rat that follow a non-genotoxic mechanism are highly specific to rodents and of no relevance for humans.

Thus, in the present case Pyrethrins with only a marginally tumorigenic activity in rodents act apparently by a mechanism similar to Phenobarbital, but the potency is about 5-10-fold lower with regard to biochemical effects. Phenobarbital has been shown to be non-carcinogenic to humans even when administered at pharmacologic levels.

The results show that Pyrethrins, in common with other non-genotoxic oncogens (phenobarbital) caused liver and thyroid gland tumours through a dose related proliferative response in the liver (replicative DNA synthesis and microsomal enzyme induction) and a secondary proliferative stimulation of thyroid follicular cells which is specific to rats. The lack of any effect of Pyrethrins at 100 ppm (6/8 mg/kg bw/d extract) confirms the threshold nature of this effect ([redacted]; Pfeil, 2003; [redacted]). (BRA, MGK and SCJ)

Mouse

Groups of 60 Charles River CD-1 mice of each sex received diets containing 0, 100, 2500, and 5000 ppm total pyrethrins for 18 months, equal to doses of 0, 14, 350, and 690 mg/kg bw/d total pyrethrins (0, 22, 530, and 1051 mg/kg bw/d extract) for males and 0, 17, 400, and 830 mg/kg bw/d total pyrethrins (0, 26, 633, and 1278 mg/kg bw/d extract) for females.

One male and one female in the 5000 ppm (1051/1278 mg/kg bw/d extract) group were found dead during the first week of the study but no further treatment related deaths occurred. All 5000 ppm (1051/1278 mg/kg bw/d extract) animals exhibited increased activity when stimulated during the first week of study only. No dose related differences in body weight gain and food consumption were observed. There were no test substance related changes in the differential blood counts.

The only possibly test substance related changes at necropsy were:

- discoloured dark livers more common in males at 5000 ppm (1051 mg/kg bw/d extract) and in females at 2500 and 5000 ppm (633 and 1278 mg/kg bw/d extract)
- increased absolute and relative liver weights in both sexes at 2500 and 5000 ppm (530/633 and 1051/1278 mg/kg bw/d extract)
- vacuolar fatty changes in the livers of males at 2500 and 5000 ppm (530 and 1051 mg/kg bw/d extract))

The lung carcinomas in males were not treatment–related since the incidences at 2500 and 5000 ppm (530/633 and 1051/1278 mg/kg bw/ extract) were similar to those in the control groups. For females there was no evidence for carcinogenic response. The NOEL/NOAEL was 100 ppm total pyrethrins, equal to 14 mg/kg bw/d total pyrethrins (22 mg/kg bw/d extract) for males and 17 mg/kg bw/d total pyrethrins (26 mg/kg bw/d extract) for females [redacted]. (KPIC and BRA, MGK and SCJ)

Table A.51 Compilation of some factors that may be taken into consideration in classification and labelling
No data are available.

A.2.9.2 Comparison with the CLP criteria

It does not meet the EU criteria to be classified as carcinogenic. There is a clear causal link between the *Chrysanthemum cinerariaefolium* extract from HCS and the incidence of benign neoplasm in liver and thyroid established in rat and mouse. However, the mechanism has been characterized and can be concluded that there is no risk to human health (CLP 3.6.2.2.3.(b)) explained in the point A3.9.1 in this CLH report.

A.2.9.3 Conclusion on classification and labelling for carcinogenicity

Not classified.

A.2.9.4 Overall conclusion on carcinogenicity related to risk assessment

Conclusion used in Risk Assessment – Carcinogenicity	
Value/conclusion	Not carcinogenic.
Justification for the value/conclusion	From the testing detailed above there are no indications of carcinogenic potential.
Proposed classification	Not classified.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two 2-year dietary studies (in rats and in mice) are described in the CLH Report. The Dossier Submitter assigned both studies with a reliability score 1 and considered them as key studies.

2-year dietary toxicity in rats (KPIC/MGK, BRA, and SCJ, 1990a)

The highest dose of *Chrysanthemum cinerariaefolium* extract (3000 ppm) caused occasional reductions in body weight gain in both sexes. At the mid-dose and high dose (1000 ppm and 3000 ppm), increased and accentuated lobulation of the liver in males was noted, and an increased activity of serum transaminases in males was observed at 3000 ppm.

Regarding neoplastic changes, there was an increased incidence of keratoacanthomas in males (3000 ppm), an increased incidence of hyperplasia and follicular cell adenomas of the thyroid (at 1000 ppm in males; at 3000 ppm in both sexes), and a slightly increased incidence of hepatic adenoma in females (3000 ppm).

The Dossier Submitter considers that the increased incidence of skin keratoacanthomas does not have any true toxicological significance due to the self-limiting nature of this lesion.

Regarding increased incidences of liver adenomas, and thyroid hyperplasia and follicular adenomas, the Dossier Submitter considers that the Applicant provided adequate justification through additional testing to demonstrate that the mechanisms by which pyrethrins cause these rodent tumours are not relevant for humans (two mechanistic studies are presented in the CLH Report: A definitive mechanistic toxicity study in rats with pyrethrins (2002) and a subsequent evaluation of liver samples from the same study (2002)). Pyrethrins showed only a marginally tumorigenic activity in rodents, apparently by a mechanism similar to phenobarbital, but with a potency about 5-10-fold lower with regard to biochemical effects. Mechanistic studies showed that pyrethrins, in common with other non-genotoxic rodent tumour promoters like phenobarbital, caused liver and thyroid gland tumours through a dose related proliferative response in the liver and a secondary proliferative stimulation of thyroid follicular cells which is specific to rats. The lack of any effect of pyrethrins at 100 ppm confirms the threshold nature of this effect.

18-month dietary oncogenicity study in mice (KPIC/MGK, BRA, and SCJ, 1990b)

At the two highest doses of *Chrysanthemum cinerariaefolium* extract, 2500 and 5000 ppm, discoloured dark livers and increased absolute and relative liver weights were observed in males and females, and vacuolar fatty changes in the livers of males only. Lung carcinomas in males were not treatment related. In females, there was no evidence of a carcinogenic response.

The Dossier Submitter concluded that *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent does not meet the CLP criteria to be classified as carcinogenic. Although, there is a clear causal link between the *Chrysanthemum cinerariaefolium* extract and the incidence of benign neoplasm in liver and thyroid established in rats, the mechanism has been characterised and can be concluded that there is no relevance to human health.

Comments received during consultation

There was a question from one MSCA on the numerical values for liver adenoma and keratoacanthoma incidences in the rat study, as well as lung carcinoma in the mouse study. The Dossier Submitter responded that these values are presented in the CLH Report, as well as in the DAR, and they have also been included in the background document (section "Supplemental information - In depth analyses by RAC").

Additional key elements

Keratoacanthomas

Keratoacanthomas (KA) derive from the squamous epithelium of the hair follicle infundibulum. In rodents, they are well demarcated with no evidence of invasion, and, as pointed out in the RAC Opinion on glyphosate (2022), they are rather common in aged male rats. In mice, it was observed that experimental keratoacanthomas can undergo spontaneous regression, most-likely caused by a non-immunological process correlated with the hair growth (Mecklenburg et al. 2013). However, some keratoacanthomas in mice can transform into squamous cell carcinomas with invasive growth into the dermis and subcutis (Mecklenburg *et al.* 2013).

In humans, KA are 1 to 2 cm dome-shaped skin tumours with a centralized keratinous plug. Multiple aetiologies for KA in humans have been suggested, most often ultraviolet radiation (RAC Opinion on glyphosate, 2022), although exposure to chemical carcinogens, immunosuppression, genetic predisposition (e.g. mutations of p53 or H-Ras), viruses (including human papillomavirus), and recent trauma or surgery at KA location, were also described (Zito and Scharf, 2022). Although most cases of KA occur on sun-exposed hair-baring areas, they may arise in areas without sun exposure (e.g. subungual areas, buttocks, and anus). KA occurs more frequently in men with a male to female ratio of 2:1 (Zito and Scharf, 2022). KA has an initial rapid growth followed by a period of variable tumour stability, and, in most cases, spontaneous regression. Although considered as a benign skin tumour prone to spontaneous involution, KA is considered a variant of squamous cell carcinoma (SCC) (it shares histopathological features with SCC), and it requires treatment. In rare cases it can invade surrounding tissues, have a perineural spread, or even metastasise (Zito and Scharf, 2022; Sisti *et al*., 2020).

A recent review points out that KA, although a common skin tumour, remains

controversial regarding classification, epidemiology, diagnosis, prognosis and management (Tisack *et al*., 2021). Newer evidence shows that several morphological, biological, molecular, and immunological characteristics indicate that KA, although related to SCC, is a separate, benign entity. However, a reliable set of criteria to discriminate between KA and SCC is still missing (Tisack *et al*., 2021).

Mechanistic studies to elucidate human relevance of liver and thyroid changes observed in rodents

In addition to two mechanistic studies presented in the CLH Report, 1) Definitive mechanistic toxicity study in rats with pyrethrins (2002), and 2) Evaluation of liver samples from the same study (2002), three further mechanistic studies are briefly described in the RAR:

3) An investigation of the effects of Pyrethrins on some cytochrome P450 forms in cultured rat and human hepatocytes (CA 5.8.2/05 Lake, 2006) (similar or possibly even the same results are described in Price *et al*., 2008);
4) An investigation of the effects of pyrethrins on some rat hepatic peroxisomal enzyme activities (CA 5.8.2/06 Lake, 2006b);
5) Examination of the mode of action of pyrethrin tumorigenesis in mammals (CA 5.8.2/07 Klaunig J, 2006; US EPA, 2008).

Also, the data from these studies were evaluated in a mode of action analyses for liver and thyroid gland tumours in the rat (e.g. Finch *et al.,* 2006; Osimitz and Lake, 2009).

1) Definitive mechanistic toxicity study in rats with pyrethrins (2002); 2) Evaluation of liver samples from the same study (2002)

These GLP studies investigated whether pyrethrins induce liver and thyroid gland changes in rats by microsomal enzyme induction. Rats, 60 per sex per group, were orally exposed to pyrethrum extract (FEK-99 blend; purity 57.03%) for up to 42 days at 0, 100 ppm (6.9-7.6 mg/kg bw/day total pyrethrins), 3000 ppm (172-214 mg/kg bw/day total pyrethrins), and 8000 ppm (277-545 mg/kg bw/day total pyrethrins) in females, and at 0 ppm and 8000 ppm (316-457 mg/kg bw/day total pyrethrins) in males. Oral exposure to phenobarbital was up to 14 days at 1200 ppm[18] in females (85-95 mg/kg bw/day) and males (80-83 mg/kg bw/day) (doses in brackets are achieved doses), in 30 rats per sex per group.

Clinical chemistry and thyroid hormone analysis, liver and thyroid histology, qualitative and quantitative assessment of cell proliferation in liver and thyroid (based on the staining index with BrdU) were evaluated. Washed microsomes from male and female liver samples were assayed for protein and total cytochrome P450 (CYP) content and activities of 7-ethoxyresorufin O-deethylase (EROD), 7-pentoxyresorufin O-dependentylase (PROD), and testosterone 7α-, 16ß- and 6ßhydroxylases (markers for induction of CYP1A, CYP2B, CYP2A, CYP2B and CYP3A forms, respectively, in rat liver), as well as for thyroxine uridine 5'-diphosphoglucuronosyl transferase (UDPGT) (which deactivates hormonally active thyroxine by forming a thyroxine glucuronide conjugate which is then excreted).

[18] The dietary concentration of phenobarbital was initially set at 1498 ppm for females and 1558 ppm for males. Because of unexpectedly severe clinical signs this was reduced on day 8 to 1200 ppm for both sexes.

Findings (please also see the tables in the section "Supplemental information - In depth analyses by RAC", background document):

Survival: Three females of the 8000 ppm feeding group were pre-maturely killed (due to the appearance of rolling gate, thin appearance, piloerection, decreased urine and faeces). All other animals survived until scheduled sacrifice.

Clinical Signs: With the exception of the three pre-mature deaths, no other treatment-related clinical signs were observed.

Body weight and food consumption: Body weight gains were statistically significantly reduced at 8000 ppm feeding group in both sexes, and very slightly at 3000 ppm in females. The decrease in body weight gain correlated with lower consumption of food.

Liver toxicity: Doses ≥ 3000 ppm increased liver weights and hepatocellular hypertrophy incidence, accompanied by evidence of increased cell proliferation.

Thyroid toxicity: Exposure to Pyrethrins resulted in increased thyroid weight and induced thyroid follicular cell hypertrophy, increased labelling with BrdU, decreased T3 and T4 levels, and increased TSH levels.

Microsomal liver enzyme analysis: Pyrethrins dose-dependently induced hepatic CYP2B and CYP3A enzymes, as well as thyroxine-UDPGT levels. CYP1A enzyme (EROD) was much less induced compared to CYP2B and CYP3A enzymes (figure in the section "Supplemental information - In depth analyses by RAC", background document).

Phenobarbital exhibited the expected response: hypertrophic changes in liver and thyroid gland (increased organ weights; histological evidence of increased cell size), increased labelling with BrdU in liver and thyroid gland, decreased T3 and T4 levels, increased TSH levels (please see the tables in the section "Supplemental information - In depth analyses by RAC", background document), we well as induction of hepatic CYP2B and CYP3A enzymes (figure in the section Supplemental information - In depth analyses by RAC", background document).

Pyrethrins and phenobarbital induced similar pattern of response in the liver and thyroid gland, but the effects were more pronounced in the case of phenobarbital.

Liver weights and hypertrophy incidence data in the definitive mechanistic toxicity study in rats with pyrethrins (2002) (copied from the DAR):

Table B.6. 102: Liver weight (g)[a] /incidence[b] of animals with histological liver cell hypertrophy in rats given diets containing pyrethrins or phenobarbital

Sex	**Dietary concentration (ppm)**	**Treatment period**			
		7 days	**14 days**	**42 days**	**42 days + 42 days recovery**
Males	0	18.09 / 0	17.66/0	21.66/0	21.77/0
	8000	23.13*** / 14	26.43***/9	30.73***/11	22.89/0
	Phenobarbital	23.21*** / 15	24.60***/15	-	-
Females	0	10.94/0	11.79/0	13.18/0	13.09/0
	100	10.77/0	11.96/0	12.35*/0	13.38/0
	3000	13.65***/11	15.00***/11	16.04***/14	13.82/0

	8000	14.70***/13	18.51***/12	18.95***/12	14.09/0
	Phenobarbital	13.12***/14	14.94***/15	-	-

[a]Using body weight as covariate
[b]No. of animals out of 15
*p<0.05,*** p<0.001

BrdU labelling index (%) in the liver in the definitive mechanistic toxicity study in rats with pyrethrins (2002) (copied from the DAR):

Table B.6. 104: Summary of BrdU labelling index (%) in liver

	Treatment group	**Treatment period**			
		7 days	**14 days**	**42 days**	**42 days + 42 days recovery**
Males	1 (0 ppm)	2.13	2.56	1.76	1.47
	4 (8000 ppm)	10.19↑	8.26↑	3.39	2.03
	5 (Phenobarbital)	25.88↑	2.47	-	-
Females	1 (0 ppm)	5.88	6.01	4.28	2.84
	2 (100 ppm)	5.68	5.37	5.62	2.72
	3 (3000 ppm)	16.74↑	16.77↑	3.71	2.08
	4 (8000 ppm)	20.60 ↑	19.28 ↑	6.54	0.80
	5 (Phenobarbital)	17.23 ↑	21.13↑	-	-

↑ indicatesindicate statistically significant increases

Thyroid weight and incidence of animals with histological thyroid follicular cell hypertrophy in definitive mechanistic toxicity study in rats with pyrethrins (2002) (copied from the DAR):

Table B.6. 103: Weight of the thyroid gland (mg)[a]/incidence[b] of animals with histological thyroid follicular cell hypertrophy in rats given diets containing pyrethrins or phenobarbital

Sex	**Dietary concentration (ppm)**	**Treatment period**			
		7 days	**14 days**	**42 days**	**42 days + 42 days recovery**
Males	0	21.8/0	23.4/0	28.2/0	30.5/0
	8000	25.6/7	29.4**/15	37.4***/13	33.9/0
	Phenobarbital	26.8*/9	29.4***/15	-	-
Females	0	19.4/0	19.8/0	22.5/0	22.9/0
	100	19.0/0	19.1/0	21.7/0	22.7/0
	3000	21.4/3	24.0**/11	25.1*/10	25.0/0
	8000	19.9 / 6	29.0*** / 14	29.8*** / 14	27.8** / 0
	Phenobarbital	20.1/0	22.6* / 5	-	-

[a]Using body weight as covariate
[b]No. of animals out of 15
*p<0.05, **p<0.01, *** p<0.001

BrdU labelling index (%) in the thyroid gland in the definitive mechanistic toxicity study in rats with pyrethrins (2002) (copied from the DAR):

Table B.6. 105: Summary of BrdU labelling index (%) in thyroid gland

	Treatment group	**Treatment period**			
		7 days	**14 days**	**42 days**	**42 days + 42 days recovery**
Males	1 (0 ppm)	2.96	2.95	3.78	2.38
	4 (8000 ppm)	3.33	11.54↑	4.86	1.82
	5 (Phenobarbital)	5.24	16.61↑	-	-
Females	1 (0 ppm)	2.65	4.96	4.47	3.53
	2 (100 ppm)	2.85	6.70	3.56	3.23
	3 (3000 ppm)	7.82	17.43↑	3.74	1.61
	4 (8000 ppm)	5.67	33.48↑	3.62	1.67
	5 (Phenobarbital)	2.44	17.14↑	-	-

↑ indicate statistically significant increases

T3 and T4 levels in definitive mechanistic toxicity study in rats with pyrethrins (2002) (copied from the DAR):

Table 6.6. 106: Serum concentrations of thyroxine/triiodothyronine (ng/mL) in rats given diets containing pyrethrins or phenobarbital

Sex	**Dietary concentration (ppm)**	**Treatment period**			
		7 days	**14 days**	**42 days**	**42 days + 42 days recovery**
Males	0	3.69/83.12	2.91/72.98	3.96/75.11	3.21/79.92
	8000	2.55***/61.62***	2.24**/65.23*	3.11**/68.18	3.63/78.07
	Phenobarbital	2.51***/71.34	2.22**/59.98***	-	-
Females	0	2.36/89.24	2.59/89.44	2.34/98.81	2.48/90.05
	100	2.78/88.03	2.13*/83.82	1.89/87.44*	2.21/89.53
	3000	2.67/83.19	2.23/86.27	2.82/84.09**	2.00/85.46
	8000	2.19/76.31	2.79/84.71	3.00*/88.90*	2.16/85.46
	Phenobarbital	1.64**/80.48	1.41***/84.63	-	-

[a]Using body weight as covariate
[b]No. of animals out of 15
*p<0.05, **p<0.01, *** p<0.001

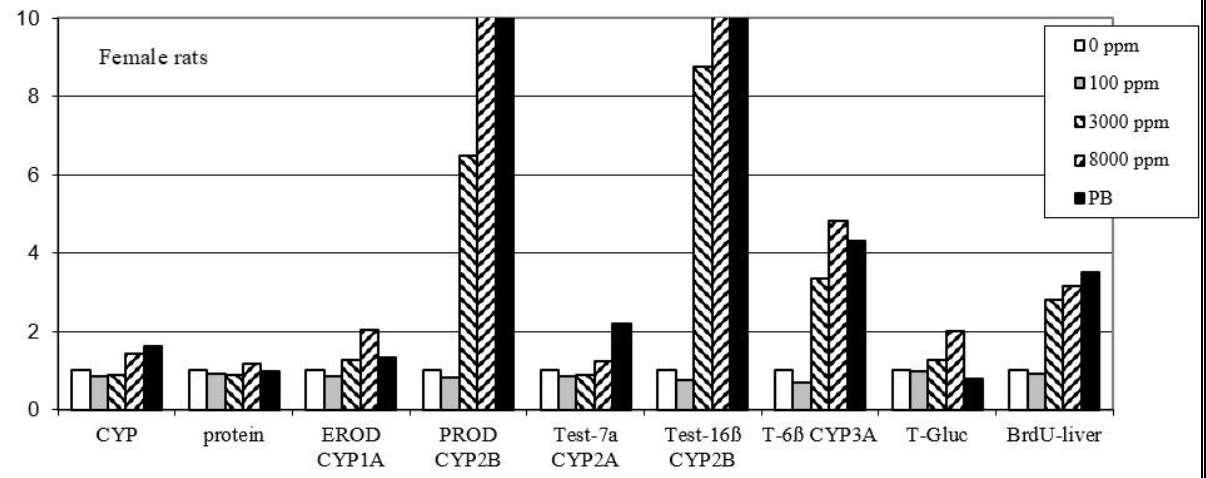
TSH levels in the definitive mechanistic toxicity study in rats with pyrethrins (2002) (copied from the DAR):

Table B.6. 107: Summary of thyroid stimulating hormone (TSH) (ng/mL)

	Treatment group	Treatment period			
		7 days	14 days	42 days	42 days + 42 days recovery
Males	1 (0 ppm)	4.26	4.46	2.68	4.50
	4 (8000 ppm)	6.28	7.69	6.95↑	2.92
	5 (Phenobarbital)	6.11	10.25	-	-
Females	1 (0 ppm)	1.87	1.97	2.07	1.86
	2 (100 ppm)	2.57	2.25	2.14	1.77
	3 (3000 ppm)	3.79↑	4.47↑	3.57	1.73
	4 (8000 ppm)	4.77↑	7.88↑	7.82↑	1.75
	5 (Phenobarbital)	4.13↑	3.99↑	-	-

↑ indicate statistically significant increases

Microsomal liver enzymes activity in the definitive mechanistic toxicity study in rats with pyrethrins (2002) (copied from the CLH Report):



Data, normalised for values of control animals, are presented for females at 14 days of treatment.

CYP = hepatic cytochrome P450 content; protein = hepatic microsomal protein content; EROD = 7-ethoxyresorufin O-deethylase; PROD = 7-pentoxyresorufin O-depentylase; Test-7a = testosterone 7α-hydroxylase; Test-16β = testosterone 16β-hydroxylase; T-6β = testosterone 6β-hydroxylase; T-Gluc = thyroxine uridine 5'-diphosphoglucuronosyl transferase (UDPGT); BrdU-liver = BrdU labelling index in liver

*3) An investigation of the effects of Pyrethrins on some cytochrome P450 forms in cultured rat and human hepatocytes (*CA 5.8.2/05 Lake, 2006*)*

The objective of this study was to obtain information on the effects of pyrethrins (FEK-99; purity: 57.03%) on cytochrome P450 (CYP) forms in rat and human hepatocytes. It was shown that pyrethrins and phenobarbital induce CYP2B and CYP3A forms in rat hepatocytes, and that pyrethrins, like phenobarbital, can induce CYP3A forms and possibly other CYP forms in cultured human hepatocytes. NOELs for induction of CYP3A4 mRNA levels and CYP3A-dependent testosterone 6ßhydroxylase activity by Pyrethrins in

human hepatocytes were 0.5 and 2 µM pyrethrins, respectively. Numerical data for rat hepatocytes are not available in the RAR, as well as further information on methodology (e.g. number of human donors).

Since limited information was available for this study in the RAR, RAC took into consideration the published study of Price *et al*. (2008), which seems to present the same results but with the methodology and results described in detail:

Effect of Pyrethrins on cytochrome P450 forms in cultured rat and human hepatocytes (Price et al., 2008)

Hepatocytes of female Sprague–Dawley rats and human (both male and female) donors were treated for 72 h with 0–1000 µM pyrethrins (Pyrethrum Extract, FEK-99 blend; purity: 57.03%) and 0–1000 µM phenobarbital. Five human donors (two male donors, 77 and 79 years old; three female donors, 60, 68 and 76 years old) were patients undergoing liver resections.
To assess the effect of pyrethrins and phenobarbital on rat hepatocyte CYP forms, the activities of 7-ethoxyresorufin O-deethylase (EROD), 7-benzyloxy-4-trifluoromethylcoumarin-*O*-debenzylase (BROD) and testosterone 6β-hydroxylase were determined, as markers of induction of CYP1A, CYP1A/2B and CYP3A, respectively. Also, mRNA levels of CYP2B1 and CYP2B1/2 were measured.
In human hepatocytes, the activities of EROD and testosterone 6β-hydroxylase were determined, as well as mRNA levels of CYP2B6 and CYP3A4.
Adequate response was obtained from positive controls. Marked cytotoxicity was not observed.
Findings: For pyrethrins, a biphasic response was generally observed both in rat and human hepatocytes: there was an initial gradual increase, maximum effect at 20 or 50 µM, then gradual decline at higher doses (an exception was CYP2B6 mRNA in human hepatocytes, which reached the maximum level at the high-dose). For phenobarbital, biphasic types of response were also observed for BROD activity, and CYP2B1 and CYP2B1/2 mRNA levels in rats, but the maximum response was observed at a one order of magnitude higher dose compared to pyrethrins. For other forms in rat hepatocytes (EROD and testosterone 6β-hydroxylase activities), and for all forms in human hepatocytes, a monotonic dose-response curve for phenobarbital was noted. It is therefore difficult to quantitatively compare the responses to pyrethrins and phenobarbital.
Comparison between human and rat hepatocytes showed the following:

- CYP1A forms: there was no EROD activity in human hepatocytes, compared to moderately increased activity in rat hepatocytes;
- CYP2B forms (mRNA level): the maximum response for CYP2B6 mRNA level in human hepatocytes was higher than for CYP2B1 and CYP2B1/2 mRNA levels in rat hepatocytes, but these occurred at an order of magnitude higher dose level than in hepatocytes from rats (200 µM *vs.* 20 µM, respectively). At 20 µM, similar increases of CYP2B6 mRNA in human hepatocytes (8.2-fold compared to control), and of CYP2B1 mRNA (7.6-fold) and CYP2B1/2 mRNA (5.1-fold) in rat hepatocytes were observed;
- CYP3A forms: testosterone 6β-hydroxylase activity was slightly lower in human hepatocytes compared to rat hepatocytes.

The results are summarised in the table below, showing a maximal increase in enzyme activity or in mRNA level.

Enzyme activity or mRNA level	**RAT HEPATOCYTES**	**HUMAN HEPATOCYTES**
EROD (**CYP1A**)	↑**2.4-fold at 20 µM PYR** ↑2.3-fold at 1000 µM PB	**no induction**
BROD (**CYP1A/2B**)	↑**2.8-fold at 20 µM PYR** ↑5.0-fold at 200 µM PB	not measured
testosterone 6β-hydroxylase (**CYP3A**)	↑**4.0-fold at 50 µM PYR** ↑2.6-fold at 1000 µM PB	↑**2.4-fold at 20 µM PYR** ↑4.2-fold at 1000 µM PB
CYP2B mRNA	CYP2B1 mRNA: ↑**7.6-fold at 20 µM PYR** ↑21-fold at 200 µM PB CYP2B1/2 mRNA: ↑**5.1-fold at 20 µM PYR** ↑29-fold at 200 µM PB	CYP2B6 mRNA: ↑**41-fold at 200 µM PYR** ↑31-fold at 1000 µM PB
CYP3A mRNA	not measured	CYP3A4 mRNA: ↑**15-fold at 50 µM PYR** ↑16-fold at 1000 µM PB

PYR = pyrethrins; PB = phenobarbital

The authors concluded that the effects of pyrethrins on the CYP-dependent enzyme activities and mRNA levels in rat and human hepatocytes were qualitatively similar to those of phenobarbital regarding the type of forms induced. Pyrethrins induced CYP2B and CYP3A forms in cultured rat and human hepatocytes, so it was demonstrated that pyrethrins are CYP2B isoform inducers (potential CAR agonists) in rat liver. The effects of both pyrethrins and phenobarbital exhibited a threshold.

*4) An investigation of the effects of pyrethrins on some rat hepatic peroxisomal enzyme activities (*CA 5.8.2/06 Lake, 2006b*)*

Liver whole homogenate samples from the original 2002 study, were assayed for protein content and cyanide-insensitive palmitoyl-CoA oxidation activity (a specific measure of the peroxisomal fatty acid ß-oxidation cycle). A relatively small increase in peroxisomal fatty acid ß-oxidation cycle enzyme at the dose level where liver tumours were observed in female rats suggests that induction of peroxisomal enzyme activities is not a key event in the mode of action of pyrethrins-induced rat liver tumours.

*5) Examination of the mode of action of pyrethrins tumorigenesis in mammals (*CA 5.8.2/07 Klaunig J, 2006; US EPA, 2008)

Rat and human hepatocytes were used to examine the effects of pyrethrins on induction of DNA synthesis (measured by incorporation of BrdU following 24-hour exposure at doses up to 150 µg/mL in female rat hepatocytes, up to 400 µg/mL in male rat hepatocytes, and up to 200 µg/mL in human hepatocytes) and inhibition of gap

junctional intercellular communication (GJIC) (by Lucifer Yellow dye coupling, following 4- and 24-hour exposures at doses up to 400 µg/mL in male and female rat hepatocytes, and up to 250 µg/mL in human hepatocytes). Potential oxidative damage by Pyrethrins was evaluated by malonaldehyde (MDA) measurement and antioxidant (vitamin E) supplementation in treated rat hepatocyte cultures. Details about human hepatocytes (number of donors, age, sex) were not available to RAC.

An inhibition of GJIC and increase in DNA synthesis by pyrethrins was observed in both male and female rat hepatocytes, but at lower concentrations in female rat hepatocytes, compared to male rat hepatocytes. Pyrethrins did not inhibit GJIC in human hepatocytes at any concentration or time point evaluated.

Cell proliferation (increase in DNA synthesis): Following 24-hour exposure, a significant increase in DNA synthesis was observed in female rat hepatocytes (up to 1.8-fold at 150 µg/mL, compared to controls) and in male rat hepatocytes (up to 1.5-fold at 400 µg/mL, compared to controls). In human hepatocytes there was no significant increase in DNA synthesis following the same exposure period.

Co-administration of 1-aminobenzotriazole (a suicide inhibitor of P450 metabolism) with pyrethrins prevented both the induction of DNA synthesis and inhibition of GJIC by pyrethrins.

Pyrethrins decreased levels of MDA, a product of peroxidation of polyunsaturated fatty acids, in rat hepatocyte cultures, and vitamin E failed to prevent the induction of DNA synthesis and the inhibition of GJIC by pyrethrins. The study author concluded that these findings indicate that oxidative damage was not involved in the effect of pyrethrins on rat hepatocytes.

Assessment and comparison with the classification criteria

Two carcinogenicity studies, one in rats and the other in mice, are briefly presented below. Mechanistic studies to elucidate the human relevance of liver and thyroid neoplastic changes observed in rodents are described in the section "Additional key elements" (background document). RAC considers that both carcinogenicity studies are sufficiently reliable for the assessment of carcinogenicity, despite their limitations (e.g. <50% survival in the control group and only one treatment group in the rat study; deviations from OECD TG 453 in both studies, stated below).

2-year dietary toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1990a)

This chronic toxicity and carcinogenicity study is a GLP compliant study, performed in line with OECD TG 453, with some deviations (haematological examination and urinalysis were not performed at 3 months; microscopic examination of the peripheral nerve was not conducted, however the sciatic nerve was examined instead; there are 2 control groups instead of 1).

Methodology

Pyrethrum Extract (FEK-99 blend; purity: 57.574%) was given at dietary levels of 0, 100, 1000 and 3000 ppm, i.e. 0, 4.6, 45 and 136 mg total pyrethrins/kg bw/day in males and 0, 5.6, 58, and 180 mg total pyrethrins/kg bw/day in females, 60 CD rats/sex/dose, for 104 weeks.

Mortality, morbidity and overt toxicity were observed daily, while more detailed clinical

observations, body weights and food consumption were measured weekly (the latter every two weeks from week 14 onwards). Ophtalmoscopic examinations were conducted once during the pretest and on all surviving rats at termination of the study. Haematology and clinical laboratory studies were performed on 15 randomly selected animals/sex/group during study months 6, 12, 18 and 24. Macroscopic and microscopic post-mortem examinations were performed on all animals.

Statistical analysis included analysis of variance with an appropriate post-hoc test (for body weights, food consumption, haematological, biochemical and urological parameters, absolute and relative organ weights). Tumour incidence data were analysed by the life table test, Hoel-Warburg "incidental tumor" test, Fischer's exact test, and Cochran-Armitage trend test.

Findings

Survival was similar among the groups (values are presented in "Supplemental information - In depth analyses by RAC", background document). No mortalities related to the test substance administration were observed. However, RAC notes that survival dropped to 40% in males and 33% in females at 100 ppm, which could affect interpretation of the results at this dose level. Survival was also low (28%) in Control 2 females, but it reached 50% in Control 1 females.

Clinical signs of toxicity were not reported.

Body weight was not affected ≤1000 ppm. At 3000 ppm body weight was (significantly) decreased during the first 78 weeks (by 6% in males and 11% in females, compared to controls, at week 78). However, later on these differences disappeared. Decrease in body weight was combined with a slight decrease in food consumption (up to 6% decrease in both sexes, compared to controls).

Haematological findings - there were no treatment related changes at any dose level (statistically significant changes were sometimes observed, however, the values were within the range of historical control data).

Clinical chemistry findings – the only change in toxicological significance was a significant increase in alanine aminotransferase (ALT) (up to 7-fold compared to control) and aspartate aminotransferase (AST) (up to 29-fold compared to control) at the 3000 ppm dose level in males.

Urinalysis - no significant treatment-related effects were noted.

Organ weights – no treatment-related effect was reported (however, thyroid, heart, spleen and uterus weights were not measured).

Macroscopic changes - accentuated lobulation of the liver was noted in male rats, with 2.5 and 2-fold increases at 1000 and 3000 ppm, respectively (values are presented in "Supplemental information - In depth analyses by RAC", background document). It is stated in the DAR that there was a correlation between this liver change and microscopic data (nature of data not specified) at dose levels of 1000 and 3000 ppm, suggesting a dose-response relationship in these dose groups.

Histopathology (values are presented in "Supplemental information - In depth analyses by RAC", background document):

Keratoacanthomas of the skin were increased by about 3-fold at the 3000 ppm dose in male rats compared to controls (Pearson's chi^2=6.52, P=0.011, compared to Control

group 1; Pearson's chi^2=5.24, P=0.022, compared to Control group 1; Pearson's chi^2=9.00, P=0.003, compared to combined control groups; calculated by RAC). The exact incidences in the mid- and low-dose groups could not be calculated since in these groups the skin was examined microscopically only if macroscopic skin changes were noted.

Thyroid hyperplasia incidence was increased at 1000 and 3000 ppm in males (8.3% and 11.7%, respectively, compared to 0 and 3.3% in controls), and at 3000 ppm in females (8.3% compared to 0 and 3.3% in controls).

Thyroid follicular adenoma was slightly increased in the 1000 and 3000 ppm males (8.3% in both treated group vs. 0% and 3.3% in two control groups) and in the 3000 ppm females (8.3% vs. 0 in control), and both male and female incidences were outside the historical control range. Relevant historical control data (same strain and laboratory, relevant time period) were in the range 0 – 7.3% (median 1.3%, interquartile range 0 – 3.3%) for males, and 0 – 4.3% (median 0%, interquartile range 0 – 1.3%) for females. The incidence of thyroid follicular carcinoma was not increased.

Altered cell foci in the liver: although the total number of foci was not increased, the histopathological severity score of the basophilic foci in female rats receiving 3000 ppm was increased (22% with moderate score out of total number of 3000 ppm females with foci) compared to the control (6% with moderate score out of total number of control females with foci (US EPA, 2008).

Liver adenoma incidence was slightly increased in 3000 ppm females (8% vs. 0% in Control 1 group and 2% in Control 2 group). Historical control data are not available.

At the highest tested dose (3000 ppm), treatment-related non-neoplastic toxicity was present as well, indicating that the MTD was achieved, but not exceeded[19]: significantly decreased body weights during the first 78 weeks (by 6% in males and 11% in females, compared to controls), slight decrease in food consumption, significantly increased ALT and AST, and accentuated lobulation of the liver in males.

RAC conclusion on the relevance of neoplastic findings in the rat study

Keratoacanthomas relevance

Incidence of keratoacanthomas (KA) was statistically significantly increased in top-dose male rats (3000 ppm) (23% *vs.* 7% in controls). The Dossier Submitter is of the opinion that due to the self-limiting nature of this skin tumour, it is unlikely that this finding has any true toxicological significance. As discussed in section "Additional key elements" (background document),", the relevance of this tumour for humans is still unclear. Recently, several morphological, biological, molecular, and immunological characteristics indicate that KA, although related to squamous cell carcinoma (SCC), is a separate, benign entity. However, a reliable set of criteria to discriminate between KA and SCC is still missing.

Liver adenomas relevance

The Dossier Submitter proposes that pyrethrins, like other non-genotoxic oncogens (e.g.

[19] According to OECD TG 453, signs of toxicity at MTD are those that may be indicated by alterations in serum enzyme levels or slight depression of body weight gain (less than 10%).

phenobarbital), caused liver and thyroid gland tumours through a dose related proliferative response in the liver and a secondary proliferative stimulation of thyroid follicular cells which is specific to rats.

The Mode of action (MoA) postulated in mechanistic studies and MoA analyses by Finch *et al*. (2006) and Osimitz and Lake (2009), is that the activation of CAR nuclear receptors in rats results in the increase in hepatic cell proliferation leading to hepatocellular tumours.

Evidence for the key events and associative events in the MoA for phenobarbital-type induction of rodent liver tumours (as proposed by Elcombe *et al*., 2014), provided for pyrethrins in mechanistic and toxicity studies, is summarised below:

Key events: CAR/PXR activation[20], leading to altered gene expression specific to CAR/PXR activation, with increased expression of CYP2B/CYP3A as associative event

- pyrethrins dose-dependently induced hepatic CYP2B (PROD, testosterone 7α- and 16ß-hydroxylases) and CYP3A enzymes (testosterone 6ß-hydroxylases) (Definitive mechanistic toxicity study in rats with pyrethrins, 2002, with subsequent evaluation of liver samples from the same study, 2002);
- pyrethrins induced CYP2B and CYP3A forms in rat hepatocytes (CYP2B1 and CYP2B1/2 mRNA, testosterone 6β-hydroxylase) and in human hepatocytes (CYP2B6 mRNA, testosterone 6β-hydroxylase) (Price *et al.,* 2008);

Key event: Transiently increased hepatocellular proliferation, with hepatocellular hypertrophy and increased liver weight as associative events

- increased liver weight and increased incidence of hepatocellular hypertrophy, accompanied by evidence of increased cell proliferation (staining index with BrdU), in male and female rats; reversibility of these changes demonstrated after 42 days of recovery (Definitive mechanistic toxicity study in rats with pyrethrins, 2002, with subsequent evaluation of liver samples from the same study, 2002);
- induction of DNA synthesis (measured by incorporation of BrdU) in male and female rat hepatocytes (Examination of the mode of action of pyrethrins tumorigenesis in mammals, 2006);
- increased liver weight (in some cases more than 60% increase, compared to controls) in short-term and sub-chronic studies in rats, mice, and dogs of both sexes (Summary tables of oral and dermal short-term studies and oral and inhalation sub-chronic studies), and in long-term study in male and female mice (18-month dietary oncogenicity study in mice, KPIC/MGK, BRA, and SCJ, 1990b), at dose levels at which treatment-related mortality was not observed;
- increased incidence of hepatocellular hypertrophy in male (2/10 vs. 0/10 in controls) and female (3/10 vs. 0/10 in controls) mice in a 90-day dietary dose range finding study (KPIC/MGK, BRA, and SCJ, 1988a);

Key event: Clonal expansion leading to altered hepatic foci

- in a 2-year dietary toxicity study in rats, altered cell foci in the liver were observed; although the total number of foci was not increased, the histopathological severity score of the basophilic foci in female rats receiving 3000 ppm pyrethrins was increased compared to the control (US EPA, 2008; Osimitz & Lake, 2009);

[20] *CAR = constitutive androstane receptor; PXR = pregnane X receptor*

Key event: Hepatocellular adenomas/carcinomas

- Liver adenoma incidence was slightly increased in top-dose females (219 mg/kg bw/day) (8% *vs.* 0% in Control 1 and 2% in Control 2).

Human non-relevance of the MoA for liver tumours

In *in vitro* studies, pyrethrins induced CYP2B and CYP3A isoforms in human hepatocytes, while EROD activity, as an indicator of CYP1A, was absent. This shows that pyrethrins can induce CAR/PXR activation in humans.

However, unlike for rat hepatocytes in which cell proliferation (assessed by increased DNA synthesis) was observed already at doses ≤150 µg/mL, pyrethrins did not induce cell proliferation in human hepatocytes up to the highest dose tested, i.e. 200 µg/mL. Also, pyrethrins did not inhibit gap junctional intercellular communication in human hepatocytes (Examination of the mode of action of pyrethrins tumorigenesis in mammals, 2006; US EPA, 2008). Unfortunately, this study was not presented in the CLH Report, while the RAR, the US EPA report from 2008, and open literature articles (such as Osimitz & Lake, 2009) do not provide details on the human hepatocyte donors (e.g. the number of donors).

Alternative MoA for liver tumours (Cohen, 2010)

ALTERNATIVE MoA	**AVAILABLE EVIDENCE**
Genotoxicity	Pyrethrins were negative in *in vitro* and *in vivo* genotoxicity studies.
PPARα receptor activation	Liver samples assayed for cyanide-insensitive palmitoyl-CoA oxidation activity showed only a small increase in peroxisomal fatty acid ß-oxidation cycle enzyme at the dose level where liver tumours were observed in female rats, which suggests that induction of peroxisomal enzyme activities is not a key event in the MoA for liver tumours (only a summary was available to RAC).
AhR receptor activation	Pyrethrins did not produce a large increase in P450 Cyp1a EROD activity in *in vivo* study in rats or in an *in vitro* study in rat hepatocytes, and no increase in EROD activity was observed in an *in vitro* study in human hepatocytes.
Estrogenic stimulation	There were no indications of estrogen-mediated adversity in repeated-dose oral and inhalation toxicity studies in rats, repeated-dose oral toxicity studies in mice and dogs, long-term toxicity studies in rats and mice, and 2-generation study in rats. Available *in vitro* mechanistic studies of pyrethrins predict no agonist or antagonist activity at the ER (RAR, 2021).
Statins	There was no evidence of periportal atypia or bile duct hyperplasia (which are known effects of statins in the rodent liver; LeBaron et al., 2014), however, liver HMG-CoA-reductase activity and CYP4A gene expression were not measured for pyrethrins.
Cytotoxicity	Marked cytotoxicity in rat and human hepatocytes was not observed in the Price *et al.* (2008) study. There was no effect on plasma total protein and bilirubin, and no increase in AST and ALT in the definitive mechanistic toxicity study in rats with pyrethrins (2002). In repeated-dose toxicity studies in rats and dogs, and in long-term toxicity study in rats, there were increases in AST and ALT, but only in male animals (while liver adenomas were observed in female rats). In *in vivo* toxicity studies there was no

	evidence of hepatic necrosis.
Immunosuppression	Toxicity studies did not indicate treatment-related effects indicative of immunotoxicity[21], however, specific testing of immune function (e.g., humoral and cell-mediated immune response) do not appear to have been performed for pyrethrins.
Porphyria	Although levels of iron or copper in the hepatocytes were not measured, there was no evidence that pyrethrins produce cell damage with regeneration in liver tissue.
Increased apoptosis	There are no data on hepatocellular apoptosis in *in vivo* or *in vitro* studies.

RAC conclusion on MoA for liver tumours

The mechanistic study *in vivo* shows pyrethrin effects on liver enzymes consistent with a CAR/PXR activator: a prominent increase in PROD and testosterone 16β-hydroxylase activity (indicating CYP2B activation), a modest increase in testosterone 6β-hydroxylase activity (indicating CYP3A activation), and a slight increase in EROD activity, which indicates only weak AhR involvement. *In vitro* studies on rat and human hepatocytes confirmed higher induction of CYP2B and CYP3A forms compared to CYP1A forms.

The lack of proliferative response in human hepatocytes suggests that the pyrethrin-induced increase in the incidence of rat liver adenomas is not relevant for humans. Nevertheless, RAC points out that the information was obtained only from secondary sources (e.g. RAR 2021, US EPA 2008) and that the details on number and characteristics of human hepatocytes' donors were not available to RAC.

RAC also notes that there are no *in vivo* studies with CAR/PXR-knock out animals or humanised-CAR animals for confirmation of CAR mediated effects. However, a study was performed in which co-administration of 1-aminobenzotriazole (inactivator of the xenobiotic metabolizing forms of cytochrome P450 metabolism) with pyrethrins prevented the induction of DNA synthesis in rat hepatocytes. Another uncertainty is that alternative MoAs have not been sufficiently investigated. For example, apoptosis cannot be excluded due to a lack of data, and epigenetic aspects were not taken into account as well.

RAC considers that the available mechanistic data indicate the **proposed MoA for liver tumours**, i.e. hepatocellular proliferation induced by activation of the CAR/PXR, **seems plausible** and **not relevant for humans**. RAC, however, points out that there are significant uncertainties in the justification for the proposed MoA and its relevance for humans, as described above.

<u>Thyroid follicular cell adenomas relevance</u>

The Dossier Submitter proposed that increased incidences of thyroid hyperplasia and follicular adenomas in rats were secondary to the induction of liver microsomal enzymes. This MoA is based on an increase in the activity of hepatic UDPG-transferase, which results in increased glucuronidation of thyroid hormones and increased excretion.

[21] In response to EFSA's request for additional data, the Applicant submitted a review of evidence relevant to address the immunotoxicity potential of pyrethrins. The review did not show treatment-related effects on the thymus or spleen organ weights, thymus, spleen, or bone marrow histopathology, or effects on the haematology parameters indicative of immunocompromised function.

Reduction in circulating T4 results in an increase of TSH, which stimulates thyroid growth manifested by follicular cell hypertrophy, hyperplasia, and neoplasia.

A mechanistic study was performed to investigate this MoA. In the *Definitive mechanistic toxicity study in rats with pyrethrins (2002)*, with *Evaluation of liver samples from the same study (2002)*, treatment with pyrethrins induced hepatic thyroxine-UDPGT levels decreased T3 and T4 levels, increased TSH levels, induced thyroid follicular cell hypertrophy, and increased thyroid weight. In this study, phenobarbital induced a similar pattern of response in the thyroid gland.

As stated in the CLP Guidance, it is known that rodents are highly sensitive to thyroid toxicity (e.g. hypertrophy, hyperplasia) after repeated stimulation of this organ, due to a reduction in thyroid hormone levels (T4). This mechanism cannot be directly extrapolated to humans, since humans, unlike rodents, possess a T4 binding protein that greatly reduces susceptibility to plasma T4 depletion and thyroid stimulation.

In support of this MoA is the observation that increased incidences of thyroid follicular adenomas were found in rats at dose levels at which liver changes were also observed (1000 ppm and 3000 in males; 3000 ppm in females), and that thyroid toxicity was not observed in non-rodent species (i.e. dogs).

RAC, however, notes that other potential MoAs, such as inhibition of thyroid peroxidase or iodothyronine deiodinases, interference with the sodium-iodide symporter, or binding to thyroid hormone transport proteins (Hurley, 1998; ECHA, EFSA ED Guidance, 2018), have not been assessed

RAC conclusion on MoA for thyroid tumours

RAC considers that the proposed MoA, which is **not relevant for humans**, seems plausible, but also notes the above stated uncertainty.

18-month dietary oncogenicity study in mice (KPIC/MGK, BRA, and SCJ, 1990b)

This chronic toxicity and carcinogenicity study is a GLP compliant study, performed in line with OECD TG 453, with some deviations (there was no evaluation of blood biochemistry parameters; Hb content, packed cell volume, total red blood cells and platelets were not measured; weight of epididymides, heart, thyroid and uterus were not recorded).

Methodology

Pyrethrum Extract (FEK-99 blend; purity: 57.574%) was given at dietary levels of 0, 100, 2500 and 5000 ppm, i.e. 0, 14.4, 361 and 715 mg total pyrethrins/kg bw/day in males and 0, 17.3, 430 and 869 mg pyrethrins/kg bw/d in females, to 60 CD-1 mice/sex/dose for 18 months.

Mortality, morbidity and overt toxicity were observed daily, while more detailed clinical observations, body weights and food consumption were measured weekly (the latter every two weeks from week 14 onwards). Haematology studies were performed on 10 randomly selected animals/sex/group during study months 12 and 18. All animals received a complete macroscopic and microscopic post-mortem examination.

Statistical analysis included analysis of variance with an appropriate post-hoc test (for body weights, food consumption, and absolute and relative organ weights). The two untreated control groups were treated independently with regard to animal selection as

well as all data collection. In the case of the 5000 ppm dosing group, the total incidence of lung tumours was compared independently to the incidences in the two control groups taking into account both the initial as well as the additional analysis of lung tissues. In the case of the 100 and 2500 ppm dosing groups, only the incidences determined during the initial evaluation of single sections of lung were compared to the corresponding initial evaluation of lung tissues from the control groups.

Findings

Survival – 2 treatment-related deaths occurred at 5000 ppm during the first week of the study, but otherwise, survival in control and treatment groups was similar during the study. Survival rate at the end of the study was ≥70% (Tables B.6.88 and B.6.89 in the DAR).

Clinical signs - all animals at 5000 ppm exhibited increased activity when stimulated by tapping on their cages, but only during the first week of study.

Body weight and **food consumption** – no treatment-related effects were observed.

Haematological findings - there were no treatment related changes.

Organ weights - increased absolute and relative liver weights of both sexes at 2500 ppm and 5000 ppm. At 2500 ppm, absolute weight in males and females increased by 25% and 23%, respectively, and relative weight by 26% and 20%, respectively. At 5000 ppm, absolute weight in males and females increased by 34% and 33%, respectively, and relative weight by 37% and 35%, respectively.

Macroscopic changes - discoloured dark livers at 2500 and 5000 ppm in females (12% and 25%, respectively, vs. 0 in controls) and at 5000 ppm in males (33% vs. 0 and 1.7% in controls) (values are presented in "Supplemental information - In depth analyses by RAC", background document).

Histopathology (values are presented in "Supplemental information - In depth analyses by RAC", background document):

Liver - vacuolar fatty change increased incidence at 2500 ppm and 5000 ppm in males (13% and 23%, respectively, vs. 1.7% in controls).

Lungs - alveolar/bronchiolar neoplasms incidence appeared to be increased at 5000 ppm in females when a single section of lung was examined. Because of the wide range of incidence of this type of tumour (historical control data not presented), additional sections of the lungs of all female mice in Control Group 1, Control Group 2 and in the highest dosage level group (5000 ppm) were examined. When these additional sections of the lungs were examined microscopically, the initial incidence in the female 5000 ppm group was no longer evident.

RAC agrees with the Dossier Submitter that the **results of this study do not indicate carcinogenic potential** of *Chrysanthemum cinerariaefolium* extract.

Comparison with the criteria

- Classification into category 1A

Since there is no information on carcinogenic potential of *Chrysanthemum cinerariaefolium* extract in humans, classification in category 1A is not supported.

- Classification into category 1B

Classification into this category is largely based on animal evidence, i.e. animal experiments for which there is **sufficient evidence** to demonstrate animal carcinogenicity (presumed human carcinogen).

Pyrethrins are not mutagenic and an increased incidence of tumours was noted only in one tested species, namely in rats, and not in mice, although non-neoplastic liver changes were observed in mice study as well (increased absolute and relative liver weights in both sexes; vacuolar fatty change in males), and MTD was achieved.

An increased incidence in three types of tumours were observed in rats:

- keratoacanthoma in top-dose males
- hepatocellular adenoma in top-dose females
- thyroid follicular cell adenoma in top-dose males and females.

Human relevance for keratoacanthomas is unclear, but it should be pointed out that:

- they were observed in only one sex of one species;
- they were most often benign and did not show malignancy in the study in rats; and
- no malignant squamous cell carcinomas were reported either in rats or mice.

Regarding liver adenomas and thyroid follicular cell adenomas:

- it is plausible that their MoA is not relevant for humans, although some uncertainties remain;
- the incidences of these benign tumours were rather low (8% *vs.* 0-2% in controls for hepatocellular adenomas; 8.3% *vs.* 0-3.3% in controls for thyroid adenomas);
- hepatocellular adenomas were observed in only one sex (females);
- there was no increase in carcinomas in these organs.

RAC considers that these data indicate only limited carcinogenic potential of pyrethrins in animals.

- Classification into category 2

A substance is placed into this category based on **limited evidence** of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

There is no information on the carcinogenic potential of *Chrysanthemum cinerariaefolium* extract from data from humans, but there is limited evidence of pyrethrin carcinogenicity in animals. Nevertheless, they are either of very low carcinogenic potential so their relevance for carcinogenicity assessment is weak (keratoacanthomas), or the incidence was rather low (8% *vs.* 0-2% in controls for liver adenomas, and 8% *vs.* 0-3% for thyroid follicular cell adenomas). Available data suggest that liver and thyroid adenomas are not relevant for humans, however, there are too many uncertainties due to lack of data to draw a firm conclusion.

Based on the weight-of-evidence, primarily considering the weak carcinogenic potential of keratoacanthomas and low incidences of hepatocellular adenomas and thyroid follicular cell adenomas, RAC concludes that **no classification for carcinogenicity** is warranted for *Chrysanthemum cinerariaefolium* extract with hydrocarbon solvents.

Supplemental information - In depth analyses by RAC

Findings in **2-year dietary toxicity in rats** (KPIC/MGK, BRA, and SCJ, 1990a):

MALES			**Pyrethrins** (ppm)		
Parameter	**Control 1**	**Control 2**	**100**	**1000**	**3000**
Survival (%)	57	50	40	65	60
Clinical chemistry					↑ AST, ↑ ALT
(Histo)pathology					
Number examined	60	60	60	60	60
LIVER					
Accentuated lobulation	6 (10)	8 (13)	11 (18)	17 (29)	14 (23)
Adenoma	6 (10)	1 (2)	0	3 (5)	3 (5)
Carcinoma	1 (2)	0	0	0	1 (2)
SKIN					
Keratoacanthoma	4 (7)	5 (8)	7 (NA)	6 (NA)	14 (23)
THYROID GLAND					
Hyperplasia	2 (3)	0	2 (3)	5 (8)	7 (12)
Follicular adenoma	2 (3)	0	3 (5)	5 (8)	5 (8)
Follicular carcinoma	0	1 (2)	1 (2)	2 (3)	2 (3)
FEMALES					
Survival (%)	50	28	33	50	53
(Histo)pathology					
Number examined	60	60	60	60	60
LIVER					
Altered cell foci - severity					↑
Adenoma	0	1 (2)	0	1 (2)	5 (8)
Carcinoma	1 (2)	0	0	0	0
THYROID GLAND					
Hyperplasia	0	2 (3)	1 (2)	1 (2)	5 (8)
Follicular adenoma	0	0	2 (3)	3 (5)	5 (8)
Follicular carcinoma	1 (2)	2 (3)	0	0	1 (2)

Data are presented as number (percent)

Macro and micropathological changes in **18-month dietary oncogenicity study in mice** (KPIC/MGK, BRA, and SCJ, 1990b):

				Pyrethrins (ppm)		
Parameter	**Sex**	**Control 1**	**Control 2**	**100**	**2500**	**5000**
LIVER	M	0	1	0	1 (2)	20 (33)

discloured	F	0	0	0	7 (12)	15 (25)
dark	M	1 (2)	1 (2)	2 (3)	8 (13)	14 (23)
vacuolar fatty change						
LUNGS						
neoplasm						
- single section	F	9 (15)	7 (12)	11 (18)	7 (12)	21 (35)
- additional section	F	20 (33)	20 (33)	-	-	24 (40)

There were 60 animals per analysis

A.2.10. Reproductive toxicity

A.2.10.1. *Sexual function and fertility*

Table A.52 Summary table of animal studies on adverse effects on sexual function and fertility

Summary table of animal studies on adverse effects on sexual function and fertility								
Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Dose levels Duration of exposure	NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility)			Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
Two generation Reproductive toxicity EPA Guideline 83-4 GLP Reliability 1 Key	Rat Charles River COBS® CD® Male and female 28 animals/sex/ dose	Pyrethrum extract (Blend FEK-99; Purity 57.574%) 0, 12, 120 and 360 mg/kg bw/d total pyrethrins (0, 18, 184, and 552 mg/kg bw/d extract)	NOAEL Parental 12 mg/kg bw/d total pyrethrins (18 mg/kg bw/d extract) for toxicity, 360 mg/kg bw/d total pyrethrins (552 mg/kg bw/d extract) for reproductive parameters	NOAEL F1 12 mg/kg bw/d total pyrethrins (18 mg/kg bw/d extract) for toxicity, 360 mg/kg bw/d total pyrethrins (552 mng/kg bw/d extract) for reproductive parameters	NOAEL F2 12 mg/kg bw/d total pyrethrins (18 mg/kg bw/d extract) for toxicity, 360 mg/kg bw/d total pyrethrins (552 mg/kg bw/d extract) for reproductive parameters	360 and 120 mg/kg bw/d total pyrethrins (552 and 184 mg/kg bw/d extract): Parental: body weight↓; F1, F2: neonatal bodyweight↓ No effects on reproductive parameters	-	[redacted] (KPIC) (BRA, MGK and SCJ) IIIA6.8.2

Table A.53 Summary table of human data on adverse effects on sexual function and fertility
No data are available.

Table A.54 Summary table of other relevant studies for sexual function and fertility
No data are available.

A2.10.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Pyrethrum extract was administered to 4 groups of 28 Charles River CD® rats sex/group at concentrations of 0, 12, 120 and 360 mg/kg bw/d total pyrethrins (0, 18, 184, and 552 mg/kg bw/d extract) over a two-generation period. Each group of 28 males and females formed the initial F0 parental generation. These animals were treated for a minimum of 77 days prior to the first of two matings. Weanlings from the F1b litters were randomly selected (28 rats/sex/group) to become parents of the F1 generation. These animals were treated for a minimum of 95 days prior to being mated twice to produce the F2a and F2b litters.

No treatment related effects were noted with respect to clinical signs, body weights or food consumption for the parental rats in the F0 generation. Body weights and food consumption for the parental rats in the F1 generation were significantly reduced at 360 mg/kg bw/d total pyrethrins (552 mg/kg bw/d extract) males/females, and slightly reduced at 120 mg/kg bw/d total pyrethrins (184 mg/kg bw/d extract) in males when compared with controls. These reductions were considered to be treatment related. Administration of pyrethrum extract at 360 mg/kg bw/d total pyrethrins (552 mg/kg bw/d extract) resulted in significantly reduced pup body weights for the male and female offspring for both matings of both generations. The mean body weight values for the pups at 120 mg/kg bw/d total pyrethrins (184 mg/kg bw/d extract) were also lower than controls during the F1b and F2a lactation periods. Reproductive performance and the other litter parameters assessed were not affected by ingestion of test diets at any level tested. Macroscopic and microscopic findings were considered to be spontaneous and/or incidental in nature and not related to administration of the test article.

The NOEL/NOAEL with respect to reproductive parameters is 360 mg/kg bw/d total pyrethrins (552 mg/kg bw/d extract) and with respect to parental and neonatal toxicity is 12 mg/kg bw/d total pyrethrins (18 mg/kg bw/d extract) ([redacted]). (KPIC and BRA, MGK and SCJ)

Weeks of Age	0 mg/kg bw/d extract (Control)		18 mg/kg bw/d extract		184 mg/kg bw/d extract		552 mg/kg bw/d extract	
	Male	Female	Male	Female	Male	Female	Male	Female
0	198 ± 12.3	145 ± 8.9	197 ± 12.7	146 ±9.1	197 ±12.8	142 ± 8.5	194 ± 12.0	148±9.0
1	250 ± 13.3	170 ± 11.7	251 ± 16.0	171± 13.3	251 ± 14.9	167 ± 8.7	245 ± 14.7	171±10.5
2	290 ± 15.9	186 ± 12.2	296± 18.3	189± 14.0	292 ± 18.3	180 ± 12.2	284 ± 16.5	187±13.2
3	329 ± 19.1	207 ± 15.9	337 ± 21.3	209± 16.3	334 ± 21.5	202 ± 16.0	323 ± 20.5	205±14.9
4	352 ± 23.0	217 ± 17.9	366 ± 24.4	220± 18.6	364 ± 26.0	215 ± 18.0	352 ± 23.1	215±16.8
5	378 ± 26.7	228 ± 17.8	390 ± 33.5	234± 21.8	389 ± 29.3	225 ± 18.5	373 ± 27.5	227±17.2
6	399 ± 29.3	235 ± 19.7	409 ± 33.3	239± 21.2	401 ± 36.2	233 ± 19.7	393 ± 28.4	237±18.2
7	419 ± 31.4	246 ± 19.7	428 ± 5.6	247± 23.5	423 ± 36.0	240 ± 22.4	415 ± 31.3	245±19.6
8	438 ± 34.4	252 ± 21.4	445 ± 37.4	255± 23.5	440 ± 38.2	246 ± 23.4	427 ± 31.7	251±20.3
9	451 ± 35.8	259 ± 22.1	460 ± 40.3	261± 24.4	457 ± 39.7	250 ± 26.8	442 ± 33.5	257±21.9
10	457 ± 38.5	260 ± 23.0	469 ± 41.9	263± 25.0	468 ± 40.4	254 ± 27.3	451 ± 34.4	260±22.3
11	470 ± 38.6	269 ± 23.2	487 ± 45.1	271± 27.9	482 ± 43.8	264 ± 28.7	465 ± 36.4	265±24.2
12a	472 ± 40.4	275 ± 35.9 (9)	487 ± 46.0	265± 36.1(11)	483 ± 45.1	265 ± 29.9 (13)	465 ± 37.3	275±24.4 (13)
13a	482 ± 42.5	278 (1)	495 ± 48.6	300± 74.2 (2)	490 ± 44.6	267 ± 13.3 (5)	474 ± 36.6	321±10.7 (3)
14a	497 ± 44.3	-	508 ± 51.3	375 (1)	500 ± 46.4	310 (1)	485 ± 39.0	-
15a	502 ± 45.9	-	514 ± 51.4	360 (1)	505 ± 46.1	-	490 ± 40.5	-
16a	514 ± 45.3	285 ± 40.2 (4)	528 ± 53.3	361(1)	518 ± 45.8	278 ± 33.1 (5)	505 ± 44.6	253 (1)
17a	518 ± 47.2	-	532 ± 54.5	-	529 ± 45.0	-	509 ± 45.2	-
18a	528 ± 46.9	294 ± 22.8 (15)	545 ± 61.2	298± 27.1(15)	537 ± 48.8	297 ± 20.4 (14)	518 ± 43.5	287±23.3 (14)
19a	534 ± 0.9	285±26.2 (27)	551 ± 62.6	282± 28.9 (27)	538 ± 49.4	276 ± 30.1 (26)	523 ± 49.0	273±25.1 (25)
20	541 ± 52.3	297 ± 26.5 (28)	559 ± 64.7	296± 8.5 (27)	549 ± 50.3	288 ± 29.7 9 28)	533 ± 49.2	279±24.5 (27)
21	548 ± 53.8	302 ± 25.5 (28)	568 ± 66.9	305 ± 32.6 (27)	557 ± 52.9	294 ± 30.4 (28)	537 ± 50.0	285 ± 24.0 (27)
22	557 ± 57.0	309 ± 27.1 (28)	580 ± 68.5	313 ± 36.1 (27)	568 ± 54.9	301 ± 33.0 (10)	546 ± 51.5	288 ± 29.0 (27)
23b	551 ± 56.3	323 ± 30.2 (13)	570 ± 67.0	329 ± 48.4 (10)	560 ± 55.1	310 ± 46.1 (10)	537 ± 53.0	294 ± 29.0 (15)
24b	557 ± 59.6	358 ± 45.8 (4)	577 ± 69.2	337 ± 66.1 (5)	567 ± 57.6	349 ± 27.8 (5)	542 ± 54.0	298 ± 8.9 (3)
25b	563 ± 57.9	352 ± 15.9 (3)	585 ± 72.9	364 ± 66.0 (4)	571 ± 59.3	392 ± 21.2 (2)	549 ± 54.9	303 ± - (1)
26b	562 ± 57.6	348 ± 6.4 2)	585 ± 71.7	403 ± 52.0 (3)	569 ± 60.4	376 ± 16.3 (2)	544 ± 52.7	-
27b	574 ± 59.6	339 ± - (1)	597 ± 75.1	427 ± 4.2 (2)	581 ± 60.8	369 ± 14.8 (2)	558 ± 58.4	-
28	590 ± 61.6	350 ± - (1)	613 ± 77.6	446 ± - (1)	596 ± 63.6	378 ± 15.6 (2)	576 ± 60.3	-
29	592 ± 63.5	354 ± - (1)	615 ± 78.1	421 ± - (1)	601 ±66	381 ± 14.8 (2)	580 ± 61.9	-

Note: Number of animals weighed was 28 unless otherwise stated in brackets of results column.

aF_{1a}= Gestation/lactation period

bF_{1b}= Gestation/lactation period

Weeks of study	0 mg/kg bw/d extract		18 mg/kg bw/d extract		184 mg/kg bw/d extract		552 mg/kg bw/d extract	
	Male	Female	Male	Female	Male	Female	Male	Female
5	152 ± 13	127 ± 10.3	154 ± 13.6	133 ± 7.7 *	145 ± 11.2	123 ± 13.6	135 ± 16.4**	121 ± 17.5
6	211 ± 16.8	157 ± 12.8	213 ± 16.2	163 ± 8.4 *	202 ± 14.9	153 ± 15.9	188 ± 22 **	154 ± 17.5
7	264 ± 20.4	177 ± 15.5	269 ± 20.3	186 ± 9.3 *	251 ± 20.0	173 ± 18.3	241 ± 23.5**	174 ± 18.0
8	319 ± 22	201 ± 18.1	324 ± 25.9	210 ± 12.4*	304 ± 21.3	191 ± 20.6	290 ± 25**	195 ± 21.7
9	364 ± 26.4	220 ± 19.0	368 ± 26.7 (27)	230 ± 14.3	349 ± 26.2	210 ± 25.8	330 ± 26.3**	210 ± 21.4
10	399 ± 28.9	232 ± 19.8	408 ± 29.9 (27)	244 ± 17.5	382 ± 30.0	224 ± 27.5	363 ± 30.4 **	221 ± 21.9
11	428 ± 31.1	243 ± 20.7	435 ± 34.3 (27)	258 ± 18.1 *	409 ± 32.1	233 ± 26.4	392 ± 30.7 **	231 ± 23.4
12	455 ± 33.1	254 ± 21.6	461 ± 38.5 (27)	268 ± 20.4	435 ± 36.3	243 ± 27.1	416 ± 30.9 **	240 ± 23.3
13	475 ± 37.7	262 ± 23.4	484 ± 42.6 (27)	278 ± 21.4 *	451 ± 39.1	247 ± 28.3	440 ± 37.3 **	247 ± 25.2
14	498 ± 38.3	271 ± 25.0	510 ± 44.4 (27)	285 ± 23.7	469 ± 41 .9*	256 ± 28.2	458 ± 33.3 **	255 ± 27.5
15	417 ± 38.3	277 ± 25.7	528 ± 45.9 (27)	292 ± 23.8	487 ± 43.7*	256 ± 26.0 *	477 ± 33.5**	259 ± 28.6 *
16	526 ± 40.1	281± 25.8	536 ± 50.4 (27)	297 ± 26.2	500 ± 44.3	264 ± 29.7	487 ± 32.9 **	262 ± 27.9 *
17a	535 ± 44.2	285 ± 27.4	549 ± 55.5(27)	306 ± 28.1*	512 ± 48.7	272 ± 30.9 (25)	496 ± 38.5**	266 ± 29.3 *
18a	551 ± 45.5	290 ± 26.4	562 ± 55.2 (27)	309 ± 32.4 (24)	531 ± 54.6	278 ± 31.5 (25)	509 ± 39.2 **	270 ± 30.3 (25)
19a	559 ± 47	289 ± 42.8	570 ± 56.7 (27)	327 ± 30.3 (10)	532 ± 52.3	309 ± 25.5 (4)	516 ± 38 **	275 ± 33.6 (4)
20a	570 ± 45.3	-	576 ± 58.3 (27)	328 ± 31.2 ((6)	544 ± 56.3	306(1)	525 ± 39.9 **	262 (1)
21a	578 ± 48.8	-	588 ± 63.7 (27)	318 ± 53.6 (3)	553 ± 55.3 (27)	299 ± 10.6 (2)	536 ± 39.4 **	265 (2)
22a	586 ± 52.1	-	593 ± 64.0 (27)	325 ± 21.0 (3)	563 ± 58.3 (27)	299 ± 10.6 (2)	542 ± 39.0 **	302 ± 8.5 (7)
23a	597 ± 54.7	299 ± 29.7 (5)	612 ± 67.8 (27)	342 ± 41.0 (5)	573 ± 61.2 (27)	296 ± 35.0 (3)	550 ± 39.8 **	278 ± 31.7 (9)
24a	606 ± 54.8	306 ± 26.7 (5)	618 ± 66.6 (27)	349 ± 45.7 (8)	582 ± 61.8 (27)	310 ± 33.6 (5)	561 ± 43.7 **	283 ± 37.1(22)
25a	618 ± 58.9	311 ± 19.3(20)	631 ± 65.5 (27)	339 ± 44.3 *(17)	592 ± 63.4 (27)	298 ± 29.2 (20)	571 ± 43.2 **	289 ± 34.0*
26a	628 ± 58.2	304 ± 24.9 (27)	641 ± 68.3 (27)	331 ± 43.0 *(24)	598 ± 63.5 (27)	291 ± 29.1 (27)	575 ± 43.1**	284 ± 31.5 **
27a	636 ± 60.99	311 ± 26.7 (27)	652 ± 70.6 (27)	338 ± 40.6 **(27)	608 ± 66.0 (27)	301 ± 31.5	583 ± 47.8 **	289 ± 31.7 *(27)
28b	646 ± 62.1	316 ± 28.3 (27)	658 ± 68.5 (27)	345 ± 44.3 *	611 ± 68.6 (27)	309 ± 34.7(24)	591 ± 43.3 ** (26)	296 ± 38.4 (26)
29b	652 ± 62.8	315 ± 27.1 (26)	667 ± 66.7 (27)	349 ± 46.8 **(26)	622 ± 65.0 (27)	315 ± 35.1(26)	594 ± 46.2 **	301 ± 37.7(11)
30b	649 ± 63.1	336 ± 30.4(7)	666 ± 71.0 (25)	360 ± 37.2(12)	618 ± 65.5 (24)	334 ± 33.4 (7)	594 ± 48.6**	299 ± 43.4 (1)
31b	656 ± 64.7 (22)	400	668 ± 74.2 (19)	384 ± 39.5 (8)	628 ± 69.9 (24)	330 ± 42.7 (3)	598 ± 52.5 **	374
32b	664±67.9	-	676 ± 75.5	426 ± 45.2 (3)	634 ± 72.6	338 ± 45.3 (2)	608 ± 49.7 **(27)	-
33b	665±67.8	-	678 ± 76.3	433 ± 0.7 (2)	628 ± 77.9	357 (1)	608 ± 53.8 **(27)	-
34b	673±68.6	-	688 ± 78.4	418 ± 0.0 (2)	644 ± 72.1	363 (1)	616 ± 53.7 **(27)	-
35b	679±71.9	-	695 ± 81.9	421 ± 4.2 (2)	650 ± 73.4	374 (1)	618 ± 52.6 **(27)	-
36b	687±72.1	-	698 ± 81.5	424± 10.6 (2)	655 ± 78.3	-	628 ± 51.8 **(27)	-
37b	690±74.0	-	703 ± 83.2	-	658 ± 81.1	-	634 ± 49.5 *(27)	-

38b	693±75.1	-	703 ± 81.2	-	661 ± 85	-	641 ± 53.4 *(23)	-
39b	692±76.3	-	698 ± 95.1	-	664 ± 86.3	-	629 ± 50.0 (16)	-

Note: Number of animals weighed was 28 unless otherwise stated in brackets of results column.
* =Significantly different from the control group; $p \leq 0.05$[1]
**= Significantly different from the control group; $p \leq 0.01$
aF_{2a}= Gestation/lactation period
bF_{2b}= Gestation/lactation period

A2.10.1.2 Comparison with the CLP criteria

Chrysanthemum cinerariaefolium extract from HCS does not meet the EU criteria to be classified for sexual function and fertility. There are not findings related to the sexual function or the fertility (CLP 3.7.2.1.1. (Table 3.7.1 a))).

A2.10.1.3 Overall conclusion on sexual function and fertility related to risk assessment

Conclusion used in Risk Assessment – Effects on fertility	
Value/conclusion	No effects were observed.
Justification for the value/conclusion	Not classified

A.2.10.2. *Developmental toxicity*

Table A.55 Summary table of animal studies on adverse effects on development

Summary table of animal studies on adverse effects on development							
Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Dose levels,	NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity)	Results, maternal/parental (e.g. corrected body weight gain, for all dose levels)	Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
Teratogenicity Test EPA 83-3 OECD 414 Day 6–15 post mating GLP Reliability 2 Key	Rat Charles River COBS® CD® rats Female 25/group	Pyrethrum extract (Blend FEK-99; Purity 57.574%) 0, 5, 25, and 75 mg/kg bw/d total pyrethrins (0, 8, 38, and 115 mg/kg bw/d extract)	NOAEL maternal toxicity >75 mg/kg bw/d total pyrethrins (115 mg/kg bw/d extract) NOAEL Teratogenicity Embryotoxicity 75 mg/kg bw/d total pyrethrins (115 mg/kg bw/d extract)	No treatment related effects	Critical effects Dams foetuses No treatment related effects	1. The test substance was administered daily from day 6 through 15 of gestation instead of from implantation to the day prior to scheduled caesarean section, as the guideline recommends. 2. Food consumption was not	[redacted] (KPIC) IIIA6.8.1/01 [redacted] (BRA, MGK and SCJ) IIIA6.8.1/1

						recorded as the guideline recommends.	
Teratogenicity Test EPA 83-3 OECD 414 Day 7–19 post mating GLP Reliability 2 Key	Rabbit New Zealand White SPF Female 16/group	Pyrethrum extract (Blend FEK-99; Purity 57.574%) 0, 25, 100 and 250 mg/kg/d total pyrethrins (0, 38, 153, and 383 mg/kg bw/d extract)	NOAEL maternal Toxicity: 25 mg/kg bw/d total pyrethrins (38 mg/kg bw/d extract) NOAEL Teratogenicity Embryotoxicity: 250 mg/kg bw/d (383 mg/kg bw/d extract)	Maternal (250 and 100 mg/kg bw/d total pyrethrins (383 and 153 mg/kg bw/d extract)) Body weight ↓ and excessive salivation and arched head	Critical effects Dams foetuses No effects on foetuses	1. The test substance was administered daily from day 7 through 19 of gestation instead of from implantation to the day prior to scheduled caesarean section, as the guideline recommends. 2. Individual body weights were recorded on gestation days 0, 7, 13, 20, 24 and 29 instead of at day 0, on the first day of dosing, at least every 3 days during the dosing period and on	[redacted] (KPIC) IIIA6.8.1/02 [redacted] (BRA, MGK and SCJ) IIIA6.8.1/2

						the day of scheduled kill as the guideline recommends. 3. Food consumption was not recorded as the guideline recommends.	

Table A.56 Summary table of human data on adverse effects on development

No data are available.

Table A.57 Summary table of other relevant studies for developmental toxicity

No data are available.

A2.10.2.1 Short summary and overall relevance of the provided information on adverse effects on development

In addition to the Two-generation reproductive toxicity study ([redacted]), two teratogenicity studies have been carried out:

Rat

In a definitive study groups of 25 mated female Charles River rats were given Pyrethrum extract suspended in 0.5% methylcellulose orally by gavage at doses of 0, 5, 25, and 75 mg/kg bw/d total pyrethrins (0, 8, 38, and 115 mg/kg bw/d extract) on days 6-15 of gestation at a volume of 3 mL/kg. On day 20 of gestation, the foetuses were removed surgically for evaluation. One female in the 75 mg/kg/d total pyrethrins (115 mg/kg bw/d extract)dose group delivered its litter on gestation day 19, although this premature delivery was not considered to be treatment related.

No animals died or were killed *in extremis* during the study, and no treatment-related clinical signs were observed. Hair loss was the most frequent noted finding and was observed among all groups, including the control. The body-weight gains of the treated groups were comparable to those of the controls during treatment. No evidence of fetotoxicity was found, and morphological examination revealed no teratogenic effects at any dose tested. The following observations were noted among the treated groups but were not considered to be treatment related: folded retina in one foetus in the control, one in the 5 mg/kg/d (8 mg/kg bw/d extract) dose group, three in the 25 mg/kg/d total pyrethrins (38 mg/kg bw/d extract) dose group and two in the 75 mg/kg/d total pyrethrins (115 mg/kg bw/d extract) dose group; microphthalmia, anophthalmia and right-sided aortic arch occurred in single incidences in the control, 5 mg/kg/d total pyrethrins (8 mg/kg bw/d extract) dose group and 25 mg/kg/d total pyrethrins (38 mg/kg bw/d extract) dose group, respectively.

The NOAEL for maternal toxicity was 75 mg/kg bw/d total pyrethrins (115 mg/kg bw/d extract), and that for developmental toxicity was 75 mg/kg bw/ d total pyrethrins (115 mg/kg bw/d extract), the highest dose tested [redacted]. (KPIC and BRA, MGK and SCJ)

Rabbit

In a definite study groups of 16 inseminated female New Zealand white SPF rabbits were randomly assigned to receive Pyrethrins at doses of 0, 25, 100 and 250 mg/kg/d total pyrethrins (0, 38, 153, and 383 mg/kg bw/d extract) orally by gavage on days 7-19 of gestation at a volume of 3 mL/kg in methylcellulose. On day 29 of gestation, the foetuses were removed surgically for evaluation.

All animals survived to the end of treatment. One doe aborted in the high-dose group and whole litter resorption occurred for an additional high-dose doe, but it is not clear if these findings were related to treatment. Maternal weight loss or reduced body weight gain during the treatment period and excessive salivation and arched head post-dose was observed in few animals of the mid- and high-dose groups. There were no treatment-related effects on foetal development including teratogenicity. The NOEL/NOAEL for maternal toxicity was 25 mg/kg bw/d total pyrethrins (38 mg/kg bw/d extract) and that for developmental toxicity was 250 mg/kg bw/d total pyrethrins (383 mg/kg bw/d extract), the highest dose tested [redacted]. (KPIC and BRA, MGK and SCJ)

	Total pyrethrins dosage level (mg/kg bw/d) Extract (mg/kg bw/d)											
	0 (Control) 0			**25 38**			**100 153**			**250 383**		
	No.	**%**	**± SD**	**No.**	**%**	**± SD**	**No.**	**%**	**± SD**	**No.**	**%**	**± SD**
Animals on study	16	-	-	16	-	-	16	-	-	16	-	-
Animals that were gravid	16	-	-	15	-	-	15	-	-	16	-	-
Animals that died	0	-	-	0	-	-	0	-	-	0	-	-
Animals that aborted near term	0	-	-	0	-	-	0	-	-	1	-	
Animals examined at Caesarean section	16	-	-	16	-	-	16	-	-	15	-	-
Nongravid	0	-	-	1	-	-	1	-	-	0	-	-
Gravid:	16	-	-	15	-	-	15	-	-	15	-	-
Does with resorption only:	0	-	-	0	-	-	0	-	-	1	-	-
Does with viable foetuses:	16	-	-	15	-	-	15	-	-	14	-	-
Viable foetuses/doe:	7.6	-	3.22	6.9	-	2.80	6.5	-	2.67	6.7	-	3.20
Postimplantation loss/doe:	0.4	-	0.73	1.0	-	1.81	1.1	-	1.16	0.7	-	1.18
Total implantations/doe:	8.1	-	3.04	7.9	-	2.33	7.5	-	2.67	7.4	-	2.90
Corpora lutea/doe:	11.3	-	3.87	11.5	-	2.68	10.9	-	2.17	11.0	-	3.76
Group mean preimplantation loss (%)[a]	-	28.3	-	-	32.3[d]	-	-	30.7	-	-	31.2[c]	-
Group mean postimplantation loss (%)[b]	-	5.4	-	-	12.7	-	-	14.2	-	-	8.2	-
Mean total body weight (grams):	39.0	-	5.56	41.2	-	7.24	42.6	-	6.42	41.8	-	8.31
Foetal sex distribution – male:	62	50.8	-	67	60.9	-	44	45.4	-	44	43.6	-
– female:	60	49.2	-	43	39.1	-	53	54.6	-	57	56.4	-

[a] Total number of corpora lutea – Total number of implantations x 100
Total number of corpora lutea

[b] Total number of implantations – Total number of viable foetuses x 100
Total number of implantations

[c] Value does not include doe with regressing corpora lutea
[d] Value does not include doe with number of corpora lutea less than number of implantations
Values of the treated groups did not differ significantly from those of the control group; p>0.05

TABLE 1. Summary of Maternal Antemortem Observations

Observation	Pyrethrin Dosage Level (mg/kg/day)							
	0 (Control)		25		100		250	
	Number	%	Number	%	Number	%	Number	%
Number of animals observed	16	100	16	100	16	100	16	100
No visible abnormalities	11	69	12	75	13	81	7	44
Aborted near term							1	6
Hair loss	2	13	1	6			4	25
No stool							2	13
Small amount of stool	4	25	2	13	2	13	3	19
Soft stool					1	6		
Ocular discharge			1	6				
Material around nose							1	6
Incisors malaligned			1	6				
Incisor missing							1	6
Excessive salivation, post-dose					1	6	2	13
Head arched backward, post-dose					1	6	3	19
Labored breathing, post-dose							2	13

A2.10.2.2 Comparison with the CLP criteria

Chrysanthemum cinerariaefolium extract from HCS is not classified for reproductive toxicity according to EU criteria due to there are not findings related to development (CLP 3.7.2.1.1. (Table 3.7.1 a))).

A2.10.2.3 Overall conclusion on effects on development related to risk assessment

Conclusion used in Risk Assessment – Effects on development	
Value/conclusion	No effects on development were observed.
Justification for the value/conclusion	Not classified

A.2.10.3. *Effects on or via lactation*

A2.10.3.1 Short summary and overall relevance of the provided information on adverse effects on or via lactation

An assay about the toxicokinetics and distribution of the *Chrysanthemum cinerariaefolium* extract from HCS [redacted] shows that the a.i. accumulates in the fatty tissue. This propertie indicates the likelihood that the substance is present in potentially toxic levels in breast milk. However, in the Two-generation reproductive toxicity study [redacted] there are no effects on or via lactation.

A2.10.3.2 Comparison with the CLP criteria

Chrysanthemum cinerariaefolium extract from HCS is not classified for reproductive toxicity according to EU criteria due to there are not findings related to effects on or via lactation, although substance is susceptible to being found in breast milk (CLP 3.7.2.1.1. (Table 3.7.1 b))).

A2.10.3.3 Overall conclusion on effects on development related to risk assessment

Conclusion used in Risk Assessment – Effects on or via lactation	
Value/conclusion	No effects on development were observed.
Justification for the value/conclusion	Not classified

A2.10.4 Conclusion on classification and labelling for reproductive toxicity

No effects were noted on reproduction. No classification required.

A2.10.5 Overall conclusion on reproductive toxicity related to risk assessment

No effects were noted on reproduction.

Conclusion used in the Risk Assessment – Reproductive toxicity	
Value	-
Justification for the selected value	No effects were noted on reproduction.
Proposed classification	Not classified.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

One two-generation reproductive toxicity study in the rat, and two teratogenicity studies, one in the rat and the other in the rabbit, were presented in the CLH Report. All were considered by the Dossier Submitter as key studies, but the two-generation reproductive toxicity study in the rat is was assigned a reliability score 1, while teratogenicity studies were assigned reliability score 2 due to deviations from OECD TG (the test substance

was administered daily from day 6 or 7 through 15 or 19 of gestation instead of from implantation to the day prior to scheduled caesarean section; food consumption was not recorded; maternal body weight measurements were less frequent than recommended in the Guideline).

Two-generation reproductive toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1989)

No treatment related effects were noted with respect to clinical signs, body weights or food consumption for the parental CD rats in the F0 generation at any dose level (0, 100, 1000, and 3000 ppm). Body weights and food consumption for the parental rats in the F1 generation were significantly reduced at 3000 ppm in males and females, and slightly reduced at 1000 ppm in males when compared with controls.

At 3000 ppm, and occasionally at 1000 ppm, significantly reduced pup body weights for the male and female offspring were observed, for both matings of both generations.

Reproductive performance and other assessed litter parameters were not affected by the treatment. Macroscopic and microscopic findings were considered to be spontaneous and/or incidental in nature and not related to administration of the test article.

The Dossier Submitter concluded that there are no indications that *Chrysanthemum cinerariaefolium* extract affects sexual function and fertility.

Effects on or via lactation

The Dossier Submitter notes that toxicokinetic studies with *Chrysanthemum cinerariaefolium* extract show that the active substance accumulates in the fatty tissue, indicating the likelihood that the substance is present in potentially toxic levels in breast milk. However, in the two-generation reproductive toxicity study in rats no effects on or via lactation were observed. Therefore, no classification for the effects on or via lactation is proposed.

<u>Definitive rat teratology study (KPIC/MGK, BRA, and SCJ, 1987a)</u>

There was no mortality, and no treatment-related clinical signs were observed in CD rats dosed at 0, 5, 26, and 78 mg/kg bw/d total pyrethrins, orally by gavage on days 6-15 of gestation. Also, there were no treatment-related effects on body weight gains, and no evidence of fetotoxicity. Morphological examination revealed no teratogenic effects at any dose tested.

<u>Definitive rabbit teratology study (KPIC/MGK, BRA, and SCJ, 1987b)</u>

All animals survived to the end of treatment at dose levels of 0, 26, 104 and 260 mg/kg bw/d total pyrethrins, orally by gavage on days 7-19 of gestation. One doe aborted in the high-dose group and whole litter resorption occurred for an additional high-dose doe, but it is not clear if these findings were related to treatment. Maternal weight loss or reduced body weight gain during the treatment period and excessive salivation and arched head post-dose was observed in few animals of the mid- and high-dose groups. There were no treatment-related effects on foetal development including teratogenicity.

The Dossier Submitter's overall conclusion on reproductive toxicity

Chrysanthemum cinerariaefolium extract obtained with hydrocarbon solvent should not be classified for reproductive toxicity according to the CLP criteria, since there are no

findings related to fertility, development, and effects on or via lactation.

Comments received during consultation

No comments were received regarding this toxicological endpoint.

Additional key elements

ED assessment of pyrethrins in the RAR (2021)

Conclusion of the assessment of EAS-modalities for pyrethrins in the RAR is: "The overall WoE based on the existing studies of pyrethrins indicates no EAS-mediated adversity due to exposure. Further, results from the available *in vitro* mechanistic studies of pyrethrins predict no agonist or antagonist activity at the ER or AR and no inhibition of the aromatase enzyme activity. Nevertheless, it is concluded that EAS-mediated adversity has not been sufficiently investigated for pyrethrins. Information regarding some sensitive endocrine parameters are missing from the existing OECD 416 study of pyrethrins (i.e., sperm parameters, oestrus cyclicity, anogenital distance, pubertal development, some organ weights/histopathology). However, life-time administration of pyrethrins to rats and mice produced no adverse effects of treatment in any organs sensitive to alterations in E, A, and S modalities; nor were there any effects of pyrethrins administration on reproductive function in rats. Despite the absence of any indications of endocrine activity through the E, A, and S-modalities, the EFSA/ECHA guidance may proposes that some additional OECD CF level 2/3 tests will be considered necessary for pyrethrins to further support the conclusion of no endocrine activity via the EAS-modalities."

Assessment and comparison with the classification criteria

In the CLH Report, one 2-generation reproductive toxicity study in rats and two teratogenicity studies, one in the rat and the other in the rabbit, are presented. In the DAR and RAR, however, range finding studies for teratogenicity studies in rats and rabbits are also available, and they are briefly described in RAC opinion.

Two-generation reproductive toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1989)

In this GLP study, performed in line with OECD TG 416, *Chrysanthemum cinerariaefolium* extract (FEK-99 blend; purity 57.574%) was administered to groups of Charles River CD rats (28/sex/dose) at the following doses: 0, 100, 1000, and 3000 ppm actual Pyrethrins (approximately equivalent to 0, 10, 104, and 313 mg/kg bw/day total pyrethrins) over a two-generation period.

Methodology

Rats in the F0 generation were treated for 77 days prior to the first period mating. Treatment continued through mating, gestation and lactation. Animals were allowed to mate a second time so that each female gave birth to two litters, F1a and F1b. The F1b offspring were selected (28 rats/sex/group) to be F1 generation parents and received the same test article/diet mixture for a minimum of 95 days prior to mating. This second generation also produced two litters F2a and F2b litters, with treatment throughout as

before.

Parental animals were observed for mortality and signs of overt toxicity twice each week, and body weights and food consumption were recorded weekly (except during the 21 day of mating period for males and females, when food consumption was not measured).

Parental females were also weighed during gestation days 0, 6, 15 and 20 and on lactation days 0, 7, 14 and 21.

The litters were examined for litter size, stillborns, live births and any gross anomalies. Females and their pups were observed at least twice daily for survival, behavioural abnormalities in nesting and nursing and presence of dead pups. Pups were weighed by sex on days 0, 4, 7 and 14 of lactation. Individual weights were recorded on lactation day 21.

Pathology: Ten rats/sex/group from the F1b and F2b pup generations and all F0 and F1 parental rats received a complete postmortem external and internal examination.

Statistical analysis: Comparisons of parental body weights and food consumption were performed by one-way analysis of variance and Dunnett's multiple comparisons. Fertility indices were compared using Pearson's chi-square test and Fisher's exact probability test. Pup survival indices were compared using the Mann-Whitney U-test. Numbers of liveborn pups per litter and mean body weights of pups were compared by one-way analysis of variance.

RAC notes that although the study was performed in line with the relevant OECD TG which was current at the time of the study, it lacks much information which should be provided in a more modern multigeneration study, such as the following: there was no evaluation of oestrous cycling in parental females; no evaluation of sperm parameters in parental males, except for those which failed to sire a litter; testes and epididymis weights were only recorded for males that failed to sire; anogenital distance was not measured; the age of vaginal opening and preputial separation was not evaluated for F1 weanlings; the number of implantation sites in the uteri of primiparous females was not evaluated; post-implantation loss was not reported for females of either generation; weights of uterus, ovaries, testes and epididymides (except for those which failed to sire a litter), prostate, seminal vesicles with coagulating glands, brain, liver, kidneys, spleen, pituitary, thyroid and adrenal glands, were not recorded in all P and F1 parental animals.

Findings

Parental toxicity

Survival was 100% for all groups, and there were no treatment-related clinical signs of toxicity, no treatment-related effects on reproductive parameters and organ weights, and no treatment-related macro- or microscopic changes in evaluated organs.

The only findings indicating parental toxicity were observed in the F1 generation (values are presented in the section "Supplemental information - In depth analyses by RAC", background document):

- **decreased body weights** during **premating**: at 3000 ppm up to 11% in males and up to 7% in females during the premating period compared to controls;
- **lower body weight gain** during **gestation/lactation**: please see detailed data in Developmental toxicity section below;
- **decreased food consumption** during **premating** period: at 3000 ppm up to

7% in males and up to 11% in females during premating period compared to controls; at 1000 ppm up to 6% in males compared to controls;

- **decreased food consumption** during **lactation** periods (F2a, F2b): please see detailed data in Developmental toxicity section below and tabulated values presented in "Supplemental information - In depth analyses by RAC" (background document).

Fertility

The treatment did not adversely affect male and female fertility index, copulatory interval, gestation length, or litter size. Testicular examination on males that failed to sire a litter did not show any abnormalities: sperm was present, mobile, and morphologically normal.

Developmental toxicity

Mean litter size, mean number of live born and stillborn, as well as survival indices were comparable to the control values in all treated groups of all generations. Macroscopic and microscopic findings were considered to be spontaneous and/or incidental in nature and not related to administration of the test article.

The only treatment-related effect was **decreased body weight in pups** at and/or during lactation (values are presented in "Supplemental information - In depth analyses by RAC", background document):

F1a offspring, males and females. At 3000 ppm, body weights in males and females at birth were lower for 6% and 5%, respectively, compared to controls. Body weights were recovering during lactation, but dropped again at day 14 in females (by 8%), and day 21 in males and females (by 14% and 13%, respectively).
At the same dose level (3000 ppm), maternal body weight was up to 4% lower compared to controls. Data on maternal food consumption during lactation period are not available.

F1b offspring, males and females. Statistically significantly lower body weights compared to controls were observed later in lactation period, i.e. on day 14 and 21 (9% lower on day 14 for both sexes, and 13% lower in males and 11% in females on day 21) at 3000 ppm, and in females at 1000 ppm (6% lower on day 14, 7% lower on day 21).
Maternal body weight values in the first week of gestation were 9% lower at 3000 ppm and 4% lower at 1000 ppm, compared to controls. Body weight data for the rest of the period of gestation and lactation is not available, as well as food consumption data.

F2a offspring, males and females. At 3000 ppm, statistically significantly lower body weight was present from birth till day 14 of lactation (12% to 15% lower in males, 14% to 16% lower in females, compared to control). However, on day 21, it reached 19% and 21% lower values in males and females, respectively, compared to controls. At 1000 ppm, body weights in both sexes were consistently lower by 5-7%, reaching statistical significance at birth in males, and on day 21 in females.
Compared to control values, maternal body weights at 3000 ppm were 6-7% lower during gestation and lactation[22], and food consumption during lactation was up to 14% lower.
At 1000 ppm, maternal body weights were lower by only 3-5%, compared to controls[1],

[22] RAC notes that for a lot of females, body weight measurements are missing during the period of gestation/lactation.

and there was no effect on food consumption.

F2b offspring, males and females. Lower body weights, compared to controls, were observed only at 3000 ppm, on day 14 (39% lower in males and 15% in females) and on day 21 (17% lower in both sexes).
Maternal body weights at the beginning of F2b gestation were 6-7% lower at 3000 ppm compared to control. Further data for F2b gestation and lactation are not available. Maternal food consumption during lactation was up to 20% lower at 3000 ppm, and up to 16% lower at 1000 ppm, compared to controls.

RAC considers that since there was no indication of serious maternal toxicity (no lethality, no pronounced decrease in maternal body weight, and no treatment-related clinical signs or macro- or micropathological changes), decreased body weights in pups at birth and during lactation, without any further indications of treatment-related effect, are not sufficiently severe findings to trigger classification for developmental toxicity. Significant changes in the pups' body weight occurred at doses at which maternal body weight and food consumption were also decreased, although to a lesser degree. A more pronounced decrease in body weights of pups compared to a decrease in body weight in their mothers could be explained by a higher dose per kg body weight in pups. It should be also pointed out that at the end of lactation, the pups start to nibble the mother's food.

Effects on or via lactation

Pyrethrins were found in human milk at very low concentrations (median 56 µg/kg fat, ranging from <LOD to 341 µg/kg fat; 53 human milk samples from 29 mothers living around the city of Basle, collected in 1998/1999) (Zehringer and Herrmann, 2001). The authors could not correlate these levels with the use of pyrethrin products in households. They pointed out that because the pyrethrins are relatively rapidly metabolised, they are not expected to accumulate in human milk.

According to the CLP Guidance, "the mere presence of the substance in the milk alone, without a strong justification for a concern to offspring, would normally not support classification for effects on or via lactation." RAC could not identify either human or animal data that would indicate a concern to offspring.

RAC is not aware of human studies evaluating the risk to infants due to exposure via breast milk.

Regarding animal data, RAC considers that the 2-generation study in the rat does not indicate toxic effects of pyrethrins on or via lactation. Namely, although body weights of pups were decreased in all matings of both generations, decreased body weight was primarily related to the later lactation period, i.e. days 14 and 21 after birth. Even when lower body weights were recorded at birth, either recovery occurred during the first week of lactation (F1a pups), or body weight depression observed already on day 0 remained rather constant until the last week of lactation (day 21 after birth), when it became more marked (F2a pups). In the opinion of RAC, these results indicate direct exposure of pups to the test substance (and not only via mother's milk), since it is known that at the end of lactation the pups start to nibble on the mothers' food.

Range finding study for rat teratology study (KPIC/MGK, BRA, and SCJ, 1987a-range finding)

In a GLP, non-guideline study, groups of 5 mated female Charles River rats were administered orally by gavage Pyrethrum Extract (Blend FEK-99; Purity 57.574%) at doses of 0, 37.5, 75, 150, 300 and 600 mg actual Pyrethrins/kg bw/day, i.e. 0, 39, 78, 156, 313, and 625 mg total pyrethrins/kg bw/day on days 6 through 15 during gestation, at a volume of 3 mL/kg in 0.5 % methylcellulose.

In dams, mortality and convulsions and/or tremors, occurred at 156, 313 and 625 mg/kg bw/day. Tremors were also observed at 78 mg/kg bw/day.
No effect on foetal or pregnancy parameters were seen at 39, 78, and 156 mg/kg/day. Due to the early deaths, it was not possible to perform uterine examinations at 313 and 625 mg/kg bw/day.

Definitive rat teratology study (KPIC/MGK, BRA, and SCJ, 1987a)

In this GLP study, performed according to OECD TG 414 with some deviations (animals were dosed on gestation days 6 through 15, and not day 6 through to one day prior to scheduled termination; food consumption was not recorded), groups of 25 female Charles River rats per group were administered orally by gavage Pyrethrum Extract (Blend FEK-99; Purity 57.574%) at doses of 0, 5, 25, and 75 mg/kg bw/day actual Pyrethrins, i.e. 0, 5, 26, and 78 mg/kg bw/day total pyrethrins, on days 6 through 15 during gestation, at a volume of 3 mL/kg in 0.5 % methylcellulose. On day 20 of gestation, the foetuses were removed surgically for evaluation.

Dams

No animals died or were killed *in extremis* during the study. There were no treatment-related clinical signs or effects on maternal body weight gain. No gross lesions were seen at necropsy of the study animals. One female in the high-dose group (78 mg/kg/day) delivered its litter on gestation day 19, but this premature delivery was not considered to be treatment related.

Offspring
No evidence of foetotoxicity was found, and morphological examination showed no teratogenic effects at any tested dose level (values are presented in "Supplemental information - In depth analyses by RAC", background document).

Range finding study for rabbit teratology study (KPIC/MGK, BRA, and SCJ, 1987b-range finding)

In a GLP, non-guideline study, groups of New Zealand White rabbits, 5 per group, were administered Pyrethrum Extract orally by gavage (Blend FEK-99; Purity 57.574%) at doses of 0, 37.5, 75, 150, 300 and 600 mg actual Pyrethrins/kg bw/day, i.e. 0, 39, 78, 156, 313, and 625 mg total pyrethrins/kg bw/day, on days 7 through 19 during gestation.

In does, mortality, tremors, convulsions, and weight loss were observed at 625 mg/kg bw/day, and weight loss and tremors were also seen at 313 mg/kg bw/day.
Foetotoxicity, expressed as high post-implantation loss, was observed at the maternally toxic dose of 625 mg/kg bw/day.

Definitive rabbit teratology study (KPIC/MGK, BRA, and SCJ, 1987b)

In this GLP study, performed according to OECD TG 414 with some deviations (e.g. number of females lower than recommended[23]; animals were dosed on gestation days 7 through 19, and not from day 6 through to one day prior to scheduled termination on gestation day 29; food consumption was not recorded; maternal body weight was measured less frequently than recommended), groups of 16 female New Zealand White rabbits per group were administered Pyrethrum Extract orally by gavage (Blend FEK-99; Purity 57.574%) at doses of 0, 25, 100, and 250 mg/kg bw/day actual Pyrethrins, i.e. 0, 26, 104, and 260 mg/kg bw/day total pyrethrins, on days 7 through 19 during gestation. Females were sacrificed on gestation day 29.

Does

Survival: No mortality was observed in the control and treatment groups.

Clinical signs: Excessive salivation, arched head and/or laboured breathing were observed post-dose for a few high-dose females on gestation day 18 or 19. Similarly, one mid-dose female exhibited excessive salivation and arched head post-dose on gestation day 19. No apparent treatment-related clinical signs were noted for the low-dose animals.

Body weight changes: During the treatment period (gestation days 7 through 19) body weight loss occurred in the high-dose group (-38 g), and reduced body weight gain relative to the control values was observed in the mid-dose group (36% lower) (values are presented in "Supplemental information - In depth analyses by RAC", background document). However, both non-adjusted and adjusted (by subtracting the uterus weights) maternal body weights at the end of the study were similar across all groups (98-100% of control values).

Pathology: There were no apparent treatment-related gross pathological changes.

Offspring

Cesarean section: There were no biologically meaningful statistically significant differences in the mean number of viable foetuses, post-implantation loss, total implantations, corpora lutea and foetal body weight or in the foetal sex distribution (values are presented in "Supplemental information - In depth analyses by RAC", background document).
One high-dose doe had a totally resorbed litter. Also, one doe in the high-dose group (260 mg/kg/day) aborted near term, on gestation day 28. Decreased defecation or its absence was observed for this animal on several days prior to abortion. No gross lesions were present at necropsy. It is unclear if these findings were treatment-related.

Malformations: There were no treatment-related or statistically significant differences in the incidence of foetal malformations (numerical data not available).

Developmental variations: No treatment-related effects were observed (numerical data not available).

[23] OECD TG 414: „Each test and control group should contain a sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy. Groups with fewer than 16 animals with implantation sites may be inappropriate."

RAC agrees with the Dossier Submitter that the available data in prenatal developmental studies do not indicate foetotoxicity or teratogenicity of pyrethrins. Even if one abortion and one resorbed litter in the high-dose group in rabbit teratogenicity study were considered treatment-related, the incidence of these findings is too low to trigger a classification for reproductive toxicity.

Comparison with the criteria

Fertility

The two-generation reproductive toxicity study in rats did not show an adverse effects on male and female fertility. However, it has to be pointed out that this is an old study, and a number of parameters were not evaluated (e.g. oestrous cycling in parental females; sperm parameters in parental males which did not fail to sire a litter; weights of reproductive organs; the number of implantation sites of primiparous females; post-implantation loss).

In other toxicity studies, the only effect observed was a decrease in testes weight in 8-week dietary study in dogs (13% decrease at 31 mg/kg bw/day, and 24% decrease at 90 mg/kg bw/day, compared to controls). Nevertheless, no adverse histopathological changes were seen in the testes of these animals, and testes weights were not affected in a 1-year dietary study in dogs.

In the assessment of endocrine disrupting properties of pyrethrins in the RAR, it is stated that despite limitations in the database, the existing studies of pyrethrins indicates no EAS-mediated adversity, and that available *in vitro* mechanistic studies of pyrethrins did not predict agonist or antagonist activity at the ER or AR and no inhibition of the aromatase enzyme activity.

RAC concludes that based on this information, classification for fertility is not warranted.

Developmental toxicity

According to CLP criteria, the major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

Human data on adverse effects on fertility, development, and effects on or via lactation, are not available.

Overall results of the two-generation study in rats and teratogenicity studies in rats and rabbits, do not indicate that *Chrysanthemum cinerariaefolium* extract causes mortality, structural abnormality or functional deficiency of the developing organism. Regarding altered growth, the only effect clearly related to treatment with pyrethrins' is reduced body weight in rat pups at birth and during lactation, observed in the two-generation study. Nevertheless, RAC considers that this adverse effect without any further indications of treatment-related effects in available reproductive toxicity studies, is not sufficiently severe to trigger the classification for developmental toxicity. As discussed above, significant changes in the pups' body weight occurred at doses at which maternal body weight was also decreased, although to a lesser degree. A more pronounced decrease in pups' body weights compared to their mothers could be explained by a higher dose per kg body weight in pups.

Effects on or via lactation

RAC also considers that the available data do not indicate the toxic effects of pyrethrins

on or via lactation.

Overall conclusion

RAC, therefore, concludes that **no classification for reproductive toxicity** is warranted for *Chrysanthemum cinerariaefolium* extract.

Supplemental information - In depth analyses by RAC

Body weights of F1 animals in a two-generation reproductive toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1989) (copied from the DAR):

Table B.6.119: Mean body weights of F1 males and F1 females in a rat two generation reproduction study with Pyrethrum Extract FEK-99 (selected weeks, n = 28)

Dosage level ppm a.i.	F1 generation sex	b.w. (g) as week of age			
		5	10	17	25
0	m	152	399	535	618
100	m	154	408	549	631
1000	m	145	382	512	592
3000	m	135**	363**	496**	571**
0	f	127	232	285	Gestation/lactation period
100	f	133*	244	306*	
1000	f	123	224	272	
3000	f	121	221	266*	

*) significantly different from the control group: $p < 0.05$
**) significantly different from the control group: $p < 0.01$

Food consumption of F1 animals in a two-generation reproductive toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1989) (copied from the DAR):

Table B.6.120: Mean food consumption of F1 males and F1 females in a rat two generation reproduction study with Pyrethrum Extract

Dosage level ppm a.i.	F1 generation sex	b.w. (g) as week of age			
		5	10	17	25
0	m	19.0	27.2	26.5	26.4
100	m	19.0	27.7	28.7	28.0
1000	m	18.1	25.7*	26.6	25.3
3000	m	16.1**	25.2**	26.1	25.8
0	f	16.5	18.3	18.8	Gestation/lactation period
100	f	17.1	18.9	19.9	
1000	f	16.1	17.8	18.1	
3000	f	15.2	16.5**	16.8*	

*) significantly different from the control group: $p < 0.05$

**) significantly different from the control group: p < 0.01

Food consumption of F1 females during lactation in a two-generation reproductive toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1989) (copied from the DAR):

Table B.6.122: Mean food consumption of F1 females during lactation in a rat two generation reproduction study with Pyrethrum Extract FEK-99

Dosage level ppm a.i.	Lactation period	g/animal/day at week			
		0-7	/-14	14-21	0-21
0	F2a	29.0	48.6	63.5	46.7
100	F2a	31.2	49.4	61.5	48.2
1000	F2a	29.2	45.8	59.7	45.1
3000	F2a	28.8	43.6**	54.4**	42.5*
0	F2b	33.6	51.1	64.0	49.6
100	F2b	31.9	47.5	59.4	45.8
1000	F2b	30.5	43.8*	53.8**	42.7*
3000	F2b	28.3*	40.8**	51.8**	40.2**

*) significantly different from the control group: p < 0.05
**) significantly different from the control group: p < 0.01

Body weights of offspring from both matings in a two-generation reproductive toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1989) (copied from the DAR):

Table B.6. 123: Effect of Pyrethrum Extract FEK-99 on group mean body weights for the offspring from both matings (a and b) of both generations (F1 and F2) in a rat two-generation reproduction study.

Live pups	Dosage ppm a.i.	Group mean b.w. (g) at lactation day									
		0		4		7		14		21	
		m	f	m	f	m	f	m	f	m	f
F1a	0	6.2	5.9	9.9	9.4	16.2	15.6	31.8	30.9	49.6	48.0
	100	6.5	5.9	10.6	9.9	17.2	16.1	32.7	31.1	51.6	48.7
	1000	6.3	5.9	10.1	9.7	16.5	15.9	31.5	30.6	48.3	45.9
	3000	5.8*	5.5	9.5	9.1	15.7	15.2	29.8	28.4*	42.8**	41.8**
F1b	0	6.9	6.1	10.1	9.6	16.5	16.1	33.1	32.1	52.9	50.7
	100	6.4	5.9	10.3	9.7	16.8	16.3	33.8	32.9	53.6	51.7
	1000	6.2	5.7	10.0	9.6	16.3	15.3	32.0	30.3*	51.0	47.2*
	3000	6.2	6.1	9.8	9.5	15.7	15.3	30.2*	29.3*	46.1**	45.1*
F2a	0	6.5	6.1	10.3	9.7	16.8	16.0	32.8	31.6	54.0	52.0
	100	6.6	6.1	10.7	10.2	17.4	16.8	34.5	33.2	56.4	54.1
	1000	6.1*	5.8	9.6	9.1	15.8	15.1	31.1	29.9	50.8	48.4*
	3000	5.7**	5.2**	9.0**	8.3**	14.4**	13.6**	27.9**	26.6**	43.7**	41.1**
F2b	0	6.3	5.8	9.7	9.0	15.9	15.0	33.1	31.5	52.2	49.7
	100	7.1**	6.6**	11.5*	10.9*	18.5*	17.4*	36.3*	35.0*	57.5*	54.9*
	1000	6.2	5.8	9.9	9.4	15.9	15.3	31.1	30.6	48.9	47.7
	3000	6.0	5.9	9.3	8.5	15.0	14.2	20.3**	26.8**	43.4**	41.1**

*) significantly different from the control group: $p < 0.05$
**) significantly different from the control group: $p < 0.01$

Cesarean section findings in the definitive **rat teratology study** (KPIC/MGK, BRA, and SCJ, 1987a) (copied from the DAR):

Table B.6. 125: Cesarean section observations

Observations [Mean +/- S.D.]	**Dose Level [mg/kg/day]**			
	0	**5**	**25**	**75**
No. Assigned	25	25	25	25
Females gravid	22	21	25	24
Maternal wastage				
# died	0	0	0	0
# sacrificed	0	0	0	0
# aborted	0	0	0	0
# early delivery	0	0	0	1
# non pregnant	3	4	0	1
Total *corpora lutea*	383	365	423	430
corpora lutea/dam	17.4	17.4	17.6	18.7
Total implantations	353	327	385	359
Total Implantation/dam	16	15.6	15.4	15.6
Total live fetuses	333	303	357	328
Live fetuses/dam	15.1	14.4	14.3	14.3
Total resorptions				
early	19	23	28	30
late	0	1	0	1
Dead fetuses	1	0	0	0
Pre implantation loss [%]	7.8	10.4	13.0	16.5
Post implantation loss [%]	5.7	7.3	7.3	8.6
mean uterus weight [g]	82.5	81.3	80.7	80.1
Sex ratio male/female	1.02	1.05	1.03	0.99
Fetal weight [g]	3.4	3.5	3.6	3.5

Summary of foetal external, visceral and skeletal **variations** in the definitive **rat teratology study** (KPIC/MGK, BRA, and SCJ, 1987a) (copied from the DAR):

Table B.6. 126: Summary of fetal external, visceral and skeletal variations

Dose Level [mg/kg/day]	**Fetuses/Litters**			
	0	**5**	**25**	**75**
No. Examined Externally	334	303	357	341
No. Examined Viscerally	166	151	177	170
Renal papilla not developed and/or distended ureter(s)	3/3	7/5	2/2	9/7
No. Examined Skeletally	168	152	180	171
Incomplete ossification of skull	3/2	3/2	3/3	4/3
Incomplete/nonossified hyoid body	14/6	8/6	4/3	5/5
Vertebrae reduced in ossification	2/2			
27 presacral vertebrae	1/1			1/1
25 presacral vertebrae				2/2
7th cervical rib	3/2		2/2	1/1
Sternebrae #5 and/or #6 unossified	43/15	43/18	51/17	47/17
other sternbrae unossified	1/1			
misaligned sternebrae			1/1	2/2
fewer than 13th rudimentary rib(s)	9/5	1/1	7/4	4/3
14th rudimentary rib(s)	9/5	1/1	7/4	4/3
reduced onossifies ischium			1/1	
Total fetuses/litters with variations	62/19	60/20	63/20	74/22

Summary of foetal external, visceral and skeletal **malformations** in the definitive **rat teratology study** (KPIC/MGK, BRA, and SCJ, 1987a) (copied from the DAR):

Table B.6. 127: Summary of fetal external, visceral and skeletal malformations

Dose Level [mg/kg/day]	**Fetuses/Litters**			
	0	**5**	**25**	**75**
No. Examined Externally	334	303	357	341
Anophthalmia		1/1		
Microphthalmia	1/1			
Folded retina	1/1	1/1	3/3	2/2
Right-sided aortic arch			1/1	
Total fetuses/litters with malformations	2/2	2/1	4/4	2/2
No. Examined Viscerally	166	151	177	170
No. Examined Skeletally	168	152	180	171

Maternal body weights in the definitive **rabbit teratology study** (KPIC/MGK, BRA, and SCJ, 1987b) (copied from the DAR):

Table B.6. 129: Effect of Pyrethrum Extract FEK-99 on group mean maternal body weight in a rabbit teratology study

Day of gestation	**Mean body weights (grams) at dosage levels (mg a.i./kg/d)**			
	0	**25**	**100**	**250**
0	3567	3614	3558	3561
7	3747	3741	3711	3747
13	3842	3849	3761	3692
20	3948	3952	3840	3709
24	3947	3968	3897	3791
29	3945	3948	3919	3866
29 Adjusted1)	3551	3523	3521	3475
Uterus weight	377	425	398	392

1) Gestation day 29 body weight minus uterus weight

Maternal and foetal parameters in the definitive **rabbit teratology study** (KPIC/MGK, BRA, and SCJ, 1987b) (copied from the DAR):

	Observation at dosage level (mg a.i./kg/d)										
	0 (Control)			**25**			**100**				
Parameter	**Nr.**	**%**	**±SD**	**Nr.**	**%**	**±SD**	**Nr.**	**%**	**±SD**	**Nr.**	
Animals on study	16	-	-	16	-	-	16	-	-	16	
Animals that were gravid	16	-	-	15	-	-	15	-	-	16	
Animals that died	0	-	-	0	-	-	0	-	-	0	
Animals that aborted near term	0	-	-	0	-	-	0	-	-	1	
Animals examined at Cesarean section	16	-	-	16	-	-	16	-	-	15	
Nongravid	0	-	-	1	-	-	1	-	-	0	
Gravid	16	-	-	15	-	-	15	-	-	15	
Does with resorption only	0	-	-	0	-	-	0	-	-	1	
Does with viable fetuses	16	-	-	15	-	-	15	-	-	14	
Viable fetuses/doe	7.6	-	3.22	6.9	-	2.80	6.5	-	2.67	6.7	
Post implantation loss/doe	0.4	-	0.73	1.0	-	1.81	1.1	-	1.16	0.7	

Total implantation/doe	8.1	-	3.04	7.9	-	2.33	7.5	-	2.67	7.4		-	2.90
Corpora lutea/doe	11.3	-	3.87	11.5	-	2.68	10.9	-	2.17	11.0		-	3.76
Group mean pre implantation loss (%) [1)]	-	28.3	-	-	32.3[4)]	-	-	30.7	-	-	31.2[3)]		-
Group mean post implantation loss (%) [2)]	-	5.4	-	-	12.7	-	-	14.2	-	-	8.2		-
Mean fetal body weight (grams)	39.0	-	5.56	41.2	-	7.24	42.6	-	6.42	41.8		-	8.31
Fetal sex distribution - male	62	50.8	-	67	60.9	-	44	45.4	-	44	43.6		-
- female	60	49.2	-	43	39.1	-	53	54.6	-	57	56.4		-

[1)] x 100 - not applicable
[2)] x 100 S.D. standard deviation
[3)] Value does not include doe with regressing corpora lutea
[4)] Value does not include doe with number of corpora lutea less than number of implantations Values of the treated groups did not differ significantly from those of the control group: $p > 0.05$

A.2.11. Aspiration hazard

No data are available.

A2.11.1 Short summary and overall relevance of the provided information on aspiration hazard

Not classified.

A2.11.2 Comparison with the CLP criteria

It does not meet the EU criteria to be classified as aspiration hazard because, according to CLP 3.10.2. (Table 3.10.1), kinematic viscosity > 20.5 mm^2/s.

A2.11.3 Conclusion on classification and labelling for aspiration hazard

Not classified.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

The Dossier Submitter concludes that according to CLP 3.10.2. (Table 3.10.1), *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent does not meet the CLP criteria to be classified as aspiration hazard because its kinematic viscosity is greater than 20.5 mm^2/s.

Comments received during consultation

No comments were received regarding this toxicological endpoint.

[During consultation of the CLH report for *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent, one MSCAOne MS suggested to consider that active substance should be classified for aspiration hazard, since Pyrethrins technical, which will be used in practice, contains solvent as a relevant impurity.

Assessment and comparison with the classification criteria

According to the CLP Regulation, a substance is classified for aspiration toxicity if reliable and good quality human evidence show the hazard or if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40°C.

In case of *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent, human data on aspiration hazard are not available, and the kinematic viscosity of the active substance, i.e. total pyrethrins, is greater than 20.5 mm^2/s, according to the CLH Report (please see "Supplemental information - In depth analyses by RAC", background document).

RAC notes that Pyrethrins technical contains more than 10% hydrotreated light petroleum distillate, which is classified as Asp. Tox. 1, H304[24]. However, the active substance, which is subject to classification, is not Pyrethrins technical, but total pyrethrins, which do not contain the solvent.

Based on the stated value of kinematic viscosity for the active substance, RAC agrees with the Dossier Submitter's proposal for **no classification for aspiration hazard**.

[24] According to the CLP Regulation, section 3.10.3.3.1., a mixture which contains a total of 10% or more of a substance or substances classified in Category 1, shall be classified for aspiration hazard.

A.2.12. Neurotoxicity

Table A.58 Summary table of animal studies on neurotoxicity

Summary table of animal studies on neurotoxicity						
Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Dose levels,	NOAEL, LOAEL	Results	Remarks (e.g. major deviations)	Reference
14 days EPA 81-8 GLP Single dose Reliability 1 Key	Rat Charles River CD 15 animal/sex group	Pyrethrum Extract (Batch Blend FEK-99/LS 92-37; Purity 57.467% (w/w)) Males: 0, 40, 125, and 400 mg/kg bw total pyrethrins; 0, 61, 192, and 613 mg/kg bw extract Females: 0, 20, 63, and 200 mg/kg bw total pyrethrins; 0, 31, 97, and 306 mg/kg bw extract Single dose	NOAEL: Males: 40 mg/kg bw total pyrethrins (61 mg/kg bw extract) Females: 20 mg/kg bw total pyrethrins (31 mg/kg bw extract) LOAEL: Males: 125 mg/kg bw total pyrethrins (192 mg/kg bw extract) Females: 63 mg/kg bw total pyrethrins (97 mg/kg bw extract)	400/200 mg/kg bw total pyrethrins (613/306 mg/kg bw extract) Males/females: Mortalities, acute neurological signs 125/63 mg/kg bw total pyrethrins (192/97 mg/kg bw extract) Males/females: Decreased motor activity in males Fine tremors in 3/15 females 40/20 mg/kg bw total pyrethrins (61/31 mg/kf bw extract) Males/females: No effects	-	[redacted] (KPIC) IIIA6.9 [redacted] (BRA, MGK and SCJ) IIIA6.9

Table A.59 Summary table of human data on neurotoxicity
No data are available.

A2.12.1 Short summary and overall relevance of the provided information on neurotoxicity

In a neurotoxicity study, groups of 15 male Sprague-Dawley rats received by gavage a 10% w/v solution of Pyrethrum extract in corn oil at doses of 0, 40, 125 and 400 mg /kg bw total pyrethrins (0, 61, 192, and 613 mg/kg bw extract), and 15 females received a 5% w/v solution of Pyrethrum extract in corn oil at doses of 0, 20, 63 or 200 mg/kg bw total pyrethrins (0, 31, 97, and 306 mg/kg bw extract).

Five males and two females at the high dose died on the day of treatment, and a variety of acute neurological signs were observed in the other animals at this dose, including tremors, urogenital area wetness, salivation, perinasal encrustation, exaggerated startle response, decreased grip strength, hind leg splay, and increased body temperature. Tremors were also observed in three females at the intermediate dose. Measurements of motor activity on the day of treatment indicated increased fine movement and decreased rearing and ambulation in animals of each sex at the high dose and decreased fine movement, rearing and ambulation in males as the intermediate dose. This is likely due to an effect of treatment and a predisposition for lower activity of this group if compared to the control group during pre-treatment evaluation.

	Males				**Females**			
Time posttreat.	**Pretreat.**	**3 h**	**7 d**	**14 d**	**Pretreat.**	**3 h**	**7 d**	**14 d**
N	15	14	10	10	15	15-12[1]	13	13
Cage posture								
Normal/awake	13	13	7	6	15	12	12	10
Normal/asleep	2	1	3	4	0	0	1	3
On side/prostrate	0	0	0	0	0	3	0	0
Cage clonic convulsions								
None	-	14	-	-	-	-	-	-
Explosive jumps	-	0	-	-	-	-	-	-
Cage fine tremors								
None	-	-	-	-	-	11	-	-
Whole body	-	-	-	-	-	4	-	-
Head	-	-	-	-	-	0	-	-
Cage coarse tremors								
None	-	1**	-	-	-	5**	-	-
Whole body	-	13	-	-	-	10	-	-
Cage palpebral closure								
Wide open	13	13	7	6	15	15	12	10
Half shut	0	0	0	0	0	0	0	0
Shut	2	1	3	4	0	0	1	3
Handling reactivity								
Reists	-	13	-	-	-	-	-	-
High resistance	-	1	-	-	-	-	-	-
Gait								
Normal	-	11	-	-	-	7**	-	-
Splayed	-	2	-	-	-	6	-	-
Hypotonic	-	1	-	-	-	0	-	-
Prostrate	-	0	-	-	-	1	-	-
Body position								
Normal	-	13	-	-	-	11	-	-

Hunched	-	1	-	-	-	0	-	-
On side	-	0	-	-	-	1	-	-
On stomach	-	0	-	-	-	2	-	-
Fine tremors								
None	-	11	-	-	-	7**	-	-
Whole body	-	3	-	-	-	7	-	-
Coarse tremors								
None	-	4**	-	-	-	7**	-	-
Whole body	-	10	-	-	-	7	-	-
Unusual behaviour								
None	-	-	-	-	-	13	-	-
Prostrate	-	-	-	-	-	1	-	-
Arousal								
Active/alert[2]	-	13	8	7	15	8	11	12
Hyperactive	-	0	0	0	0	1	1	0
Inactive/alert	-	1	2	3	0	3	1	1
Inactive/Not alert	-	0	0	0	0	2	0	0
Palpebral closure								
Wide open	-	-	-	10	-	-	-	-
Slightly dropping	-	-	-	0	-	-	-	-
Defecation								
None	8	9	7	9	14	13	12	11
Normal	7	4	3	1	1	1	1	2
Soft	0	1	0	0	0	0	0	0
Urine								
None	6	4	7	6	3	10	9	7
Present	9	10	3	4	12	4	4	6
Rears (events)								
Mean	7.27	2.79	5.40	4.40	6.47	3.57	8.08	9.46
SD	2.71	2.16	4.03	2.95	3.54	4.89	5.95	5.58
Approach response								
Noticeable	14	13	-	-	-	9	-	-
None	0	0	-	-	-	1	-	-
Exaggerated	1	1	-	-	-	3	-	-
Startle response								
Noticeable	12	5**	-	-	15	6**	-	-
None	0	0	-	-	0	1	-	-
Exaggerated	3	9	-	-	0	6	-	-
Tail pinch response								
Noticeable	13	10	10	9	15	11	12	-
None	0	2	0	1	0	2	0	-
Exaggerated	2	2[3]	0	0	0	0	1	-
Pupil size								
Normal	-	13	10	10	-	12	8	7
Decreased	-	1	0	0	-	1	5	6
Visual placing								
Present	-	-	10	-	-	-	13	-
None	-	-	0	-	-	-	0	-
Muscle tone								
Normal	-	11	10	-	-	11	12	-
Decreased	-	3	0	-	-	2	1	-
Fur appearance								

Normal	-	12	-	-	-	-	-	-
Urine stains	-	2	-	-	-	-	-	-
Lacrimation								
None	-	-	-	-	-	12	-	-
Present	-	-	-	-	-	1	-	-
Salivation								
None	-	-	-	-	-	12	-	-
Excessive	-	-	-	-	-	1	-	-
Crust								
None	13	9*	10	-	-	8*	12	-
Eyes	2	0	0	-	-	1	0	-
Nose	0	5	0	-	-	4	1	-
Grip strength (fore) (kg)								
Mean	0.53	0.59	0.84	0.89	0.5	0.48**	0.77	0.75
SD	0.08	0.18	0.14	0.17	0.11	0.15	0.16	0.18
Grip strength (hind) (kg)								
Mean	0.44	0.46**	0.63	0.58	0.39	0.44	0.52	0.47
SD	0.12	0.13	0.11	0.07	0.08	0.09	0.09	0.11
Rectal temperature (ºC)								
Mean	38.05	39.30**	38.17	37.95	38.06	39.68**	38.38	38.45
SD	0.05	0.57	0.57	0.47	0.46	0.43	8.08	0.63
Air righting								
Feet/Coordinated	-	-	-	10	-	-	-	-
Feet/Uncoordinated	-	-	-	0	-	-	-	-
Hind leg splay (cm)								
Mean	8.26	6.83*	8.77**	7.85	6.07	5.66	6.93	6.90
SD	1.17	1.28	1.11	1.44	0.85	1.14	1.28	1.23

*Significantly different from their respective control groups ($p < 0.05$).
**Significantly different from their respective control groups ($p < 0.01$).
[1]One animal died following cageside observations and two animals were not fully evaluated in the open field or the manipualtive portions of the FOB due to the deteriorating condition of the animals.
[2]Not significantly different from their respective control groups when evaluated as noticeable or none vs. exaggerated.
- The incidence for these endpoints was zero or the same for all doses.
In addition to the above observations, all animals were observed for the following endpoints: cage tonic convulsions, excessive vocalization, breathing pattern, clonic convulsions, tonic convulsions, palpebral closure, piloerection, exophthalmus, emaciation, and dehydration.

	Males				**Females**			
Time posttreat.	**Pretreat.**	**4 h**	**7 d**	**14 d**	**Pretreat.**	**4 h**	**7 d**	**14 d**
N	15	12	10	10	15	13	13	13
Fine movement								
Mean	294.40	657.60*	320.90	317.50	361.90	862.3*	456.70	473.80
S.D.	74.82	512.65	103.16	94.95	149.27	759.94	180.14	116.20
Ambulation								
Mean	198.90	157.90*	169.70	167.60	204.90	146.90*	268.20	272.20
S.D.	52.51	63.06	48.85	70.68	98.33	81.84	108.81	97.19
Rearing								
Mean	113.90	62.00*	128.70	136.30	137.50	66.00**	169.00	201.90
S.D.	31.42	52.55	35.20	38.90	59.98	48.11	64.52	77.28

*Significantly different from their respective control groups ($p < 0.05$).
**Significantly different from their respective control groups ($p < 0.01$).

In addition, slight, statistically non-significant decreases in body weight were seen in males at the high dose on days 7 and 14. There was no evidence of any gross, treatment-related lesion. The microscopic changes were limited mainly to sections of the sciatic nerve and its branches. The histomorphological changes within the peripheral nerve sections indicated the presence of scattered degenerating nerve fibres or myelin sheaths. These changes were seen in only a few animals, were graded as minimal, and were not dose-related.

	MALES				FEMALES			
FEEDING LEVEL (mg/kg diet)	**0**	**40**	**125**	**400**	**0**	**20**	**63**	**200**
NUMBER IN GROUP	15	15	15	15	15	15	15	15
SCIATIC NERVE								
myelin degeneration/myelin sheath swelling	0	0	0	2	0	0	0	1
minimal focal	0	0	0	2	0	0	0	1
myelin/axon degeneration	0	2	1	1	0	0	0	4
minimal focal	0	2	1	1	-	-	-	-
minimal multifocal	0	1	1	1	0	0	0	3
moderate multifocal	0	1	0	0	0	0	0	1
NERVE PERONEAL								
myelin/axon degeneration	-	-	-	-	0	1	0	2
minimal multifocal	-	-	-	-	0	1	0	1
moderate multifocal	-	-	-	-	0	0	0	1
NERVE TIBIAL								
myelin/axon degeneration	-	-	-	-	0	0	0	2
minimal multifocal	-	-	-	-	0	0	0	1
moderate multifocal	-	-	-	-	0	0	0	1

The NOEL/NOAEL was 40 mg/kg bw total pyrethrins (61 mg/kg bw extract) for male rats and 20 mg/kg bw total pyrethrins (31 mg/kg bw extract) for female rats ([redacted]). (KPIC and BRA, MGK and SCJ)

A2.12.2 Comparison with the CLP criteria

Not classified.

A2.12.3 Conclusion on neurotoxicity related to risk assessment

Conclusion used in Risk Assessment – Neurotoxicity	
Value/conclusion	Not classified
Justification for the value/conclusion	Refer to Section A3.12.1.

A.2.13. Immunotoxicity

No data are available.

A.2.14. Endocrine disruption

A2.14.1 Short summary and overall relevance of the provided information on endocrine

disruption

To evaluate a potential concern for endocrine diruption effects induced by *Chrysanthemum cinerariaefolium* extract from HCS all available data were assessed for all levels of the OECD Conceptual Framework (Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009).

A.2.14.1. *Level 1*

Existing data and non-test information, covered, physical and chemical properties, toxicological data from standardized or not-standardized tests, and read-across, chemical categories, QSARs and another *in silico* predictions, and ADME model predictions.

A few endocrine-related mechanistic studies were identified for pyrethrum flower extract from the published literature. These include:
• Several *in vitro* study of hepatic enzyme induction in rat and human hepatocytes (Price *et al.*, 2007; Price *et al.*, 2008; Osimitz & Lake, 2009).
• An *in vitro* study of human androgen receptor (AR) binding and sex hormone binding globulin (SHBG) binding (Eil and Nisula, 1990).
• An *in vitro* study of antagonistic transactivation activity at the human AR (Vinggaard *et al*., 2008).
• An *in vitro* study of agonist and antagonist activity at the human AR and the human estrogen receptors (ER)-α and -β (Kojima *et al*., 2004).

This information explains the effects of *Chrysanthemum cinerariaefolium* extract from HCS in the thyroid gland as a secondary effect of the hepatic enzyme induction.

QSAR predictions using Derek Nexus software do not trigger any alert related with endocrine-disruptive properties.

A.2.14.2. *Level 2*

In vitro assays providing data about selected endocrine mechanism(s) and pathway(s).

ToxCast models and CERAPP do not give information about estrogen- and androgen-modalities. COMPARA displays information about pyrethrin 1, pyrethrin 2, cinerin 1, and cinerin 2. These compounds are inactive as agonist for androgen receptor and active as antagonist and binding (cinerin 2 was inactive for binding too). However, these results are not correlated with results in the studies of EDSP21 database, where those positive responses take place exceeding cytotoxic limits or with an efficacy < 50%.

Steroidogenesis-modality was negative in the EDSP21 database for the only one aromatase inhibition assay.

Thyroid-modality is more ambiguous due to the solvent used in the mix. The mechanism by which the *Chrysanthemum cinerariaefolium* extract from HCS shows effects in the thyroid gland is well known, so those effects observed *in vitro* could be attributed to BHT.

A.2.14.3. *Level 3*

In vivo assays providing data about selected endocrine mechanism(s) and pathway(s).
No information on such *in vivo* assays is available for *Chrysanthemum cinerariaefolium* extract from HCS.

Mammals	Information available in the current dossier
E, A modalities	
Uteroptrophic bioassay in rodents (OECD TG 440)	N

Hershberger bioassay in rats (OECD TG 441)	N

A.2.14.4. *Level 4*

In vivo assays providing data on adverse effects on endocrine relevant endpoints.

Mammals	Information available in the current dossier
T-modality	
Repeated dose 28-day study (OECD TG 407)	N
Repeated dose 90-day study (OECD TG 408)	Y
Repeated dose 90-day study in non-rodents (OECD TG 409)	Y
Combined chronic toxicity and carcinogenicity studies (OECD TG 451-3)	Y

The thirteen-week dose range finding study in rats (OECD TG 408) has some organ weights (epididymis, prostate and seminal vesicle with coagulating glands and uterus) and histopathologies (mammary gland, prostate and seminal vesicle with coagulating glands and uterus) missing. Although some key endopoints are not available in this study, those that are on it can complement other studies because the duration of this assessment is enough. None of the organ weight changes could be correlated with microscopic findings. The liver weight changes are possibly treatment related, since some of the livers were macroscopically enlarged. Treatment related macroscopic findings included enlargement and congestion of the liver in both sexes. This was more pronounced in the male than in the female rats. These macroscopic findings in the liver could not be confirmed microscopically. All other macroscopic findings were considered to be incidental. The only possible treatment related microscopic findings consisted of small focal or multifocal areas of tubular degeneration and regeneration in the renal cortex. However, due to the low incidence of this renal lesion, it was not possible to determine its relationship with the administration of pyrethrum extract. A small number of other microscopic findings were observed in various organs at the different dosage levels, but they were considered to be incidental and not treatment related ([redacted], 1988b).

The eight-week dose range finding toxicity study in dogs (OECD TG 409) has some organ weights (epididymis and uterus) and a histopathology (seminal vesicle) missed. This study complements the others using a different mammalian target. A small number of macroscopic lesions were observed in all treated groups, but were considered not to be related to the administration of pyrethrum extract. Also, the number of animals per group is too low and this does not let perform a statistical study ([redacted], 1988a).

The one-year chronic toxicity study in dogs (OECD TG 452) has some organ weights (uterus, epididymis) and a histopathology (vagina) missed. However, these endpoints are present in other studies and the duration of this study lets see the evolution of the others endpoints clearly. About the histopathological changes in different organs related with EAS-modality the fact is the same as the other studies, the control group shows the same incidence as the treated groups, so an endocrine-related mechanism could be discarded ([redacted], 1990a).

The two-year dietary toxicity and oncogenicity study in rats (OECD TG 453) has some organ weights (uterus and epididymis) missing. However, these endpoints are present in other studies and the duration of this study lets see the evolution of the others endpoints clearly. This study shows a great amount of histopathological changes and types of tumors that could suggest estrogen-, androgen- and steroidogenesis-related mechanisms. However these effects are present in the two control groups and the difference is not statistically significant, and they are also present in other organs that are not related with endocrine disruption. Due to at this widespread incidence, it seems

that there is not an endocrine-disruption mechanism involved ([redacted], 1990b).

The eighteen-month dietary oncogenicity study in mice (OECD TG 453) has some organ weights (uterus, ovary, epididymis and adrenal glands) missed. However, these endpoints are present in other studies and the duration of this study lets see the evolution of the others endpoints clearly. About the histopathologic changes in ovary, uterus, testes, epididymis and the other organs related with estrogen-, androgen- and steroidogenesis-mediated activity, the case is that those changes are more present in these organs, but the incidence is not very different in comparison with the two control groups. This fact and the absence of statistically significant changes or the lack of the weight of some organs make impossible to assert a mechanism related with endocrine disruption. Finally, the endpoints potentially sensitive to, but not diagnostic of, E, A, T, S have the same point. The histopathologic changes are present but are very similar in comparison with the two control groups. For these reasons, this study seems to indicate a non-related mechanism because the biological significance is relative ([redacted], 1990c).

A.2.14.5. *Level 5*

In vivo assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism.

Mammals	Information available in the current dossier
EAST-modality	
Two-generation reproduction toxicity study (OECD TG 416)	Y

In the two-generation reproduction toxicity study (OECD TG 416), in general terms, there are not significant dose- or develop-related and histopathology-supported changes so this study does not show endocrine related effects or adversity in EAS-modality. Changes in adrenal glands weight could be a systemic toxicity related effect, but in any case there are not coherence between studies or between sexes. However, this study has some endpoints missing: change in AGD in pups, changes in estrus cyclicity in P and F1 generations, decreased age at VO and increased age at PPS in F1, changes in some organ weights (uterus, ovaries, testes (except for those of the males which did not sire a litter), epididymides, prostate, seminal vesicles (+ coagulating glands), thyroid and liver), some histopathologic changes (coagulating glands and thyroid) and sperm parameters (except for those of the males which did not sire a litter (numerical data not shown in the study)) in P and F1 generations. ([redacted], 1989).

A2.12.2 Comparison with the CLP criteria

Not classified.

A2.12.3 Conclusion on endocrine disruption related to risk assessment

Conclusion used in Risk Assessment – Endocrine disruption	
Value/conclusion	Not classified
Justification for the value/conclusion	Some concerns remain after the assessmet of all relevant available information of endocrine disrupting properties for *Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents. EAS-modalities have been considered sufficiently investigated and no ED properties have been detected assessing all the gathered information.

	However, T-modality has not been considered sufficiently investigated and ED properties for this modality cannot be totally excluded. Since new studies cannot be requested due to this dossier is a backlog dossier a conclusion cannot be reached. The first draft CARs for this active substances (not redefined yet) were submitted to COM before September 2013. According to the agreement reached in the CA-meeting in March 2018 (CA-March18-Doc.7.3a-Final – EDs - Active substances under assessment) the applicant is not obliged to provide new studies, although they have the opportunity to do it. In consequence, the eCA does not need to reach a conclusion regarding this issue. For EAS-modalities, although several endpoints are missing in the two-generation reproduction toxicity study, overall, the available toxicological data on repeated dose toxicity studies does not indicate EAS adversity by the test substance, suggesting that the ED criteria for EAS modalities are not met. Literature, *in silico* and *in vitro* mechanistic ToxCast data were also collected and did not indicate EATS-mediated activity with the extract individual compounds. Therefore, and although the dataset seems not complete for EAS- modalities, in the absence of adversity and activity related to the endocrine system in the organs of both genders of different species and with different time of exposure duration (short-, long term and develop/reprotoxicity studies), no further studies (especially *in vivo*) should be required to conclude on EAS-modality. However, since a new OECD TG 416 or OECD TG 443 and a literature review will be requested in the renewal stage, the chosen scenario is 2a (ii). For T-modality, as a whole, data suggest that any thyroid effects of pyrethrum flower extract are species-specific (i.e., only seen in the rat) and occur with chronic exposure only and secondary to the induction of liver microsomal enzymes. However, the potential for T-mediated adversity is considered to have not been sufficiently investigated. Overall, there is not sufficient weight of evidence to indicate that pyrethrum flower extract affects the thyroid modality by a mode of action that is specific to the rat and, as such, it cannot be concluded there are no indications of endocrine adversity of relevance to humans. Since a new OECD TG 416 or OECD TG 443 and a literature review will be requested in the renewal stage, the chosen scenario is 2a (iii).

Table 5: High level summary of the scenarios, including the next steps in the assessment; for a full description of the scenario, refer to Section 3.4.4

Adversity based on 'EATS-mediated' parameters	Positive mechanistic OECD CF level 2/3 test	Scenario	Next step of the assessment
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is no 'EATS-mediated' adversity
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis (postulate and document the MoA, see Section 3.5.1)
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis; additional information may be needed for the analysis
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no endocrine activity has been observed for the EATS modalities
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing 'EATS-mediated' parameters. Depending on the outcome of these tests move to the corresponding scenario
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis (postulate and document the MoA, see Section 3.5.1)

EATS: Estrogen, androgen, thyroid, steroidogenic; MoA: Mode of action.

A.2.15. Further Human data

Medical surveillance on manufacturing plant personnel

58 people who had been employed in a Pyrethrum extract factory for 1 to 25 years were systematically examined on digestive, cardiovascular, respiratory, muscular and endocrine systems and on sense organs. Laboratory measurements were conducted with regard to haematology, blood electrolytes, blood sugar, serum transaminases, plasma testosterone and plasma thyroxine.

The individuals were found to be clinically healthy regardless of the degree of exposure to Pyrethrins. Minor pleural lesions of inflammatory type were found in people exposed to high concentrations of aerial Pyrethrum dust (daily concentrations of 6000 ppm of Pyrethrum powder, equivalent to 78 ppm Pyrethrum extracts). These lesions did not impair the health of the individuals and were probably a reaction to irritant dust. However, the study is not acceptable and it is not possible to obtain conclusions because the original Document IV is not complete. Specifically all tables containing original data with current and previous ailments, results of the examination of the respiratory and cardiovascular systems and results of haematology and hormonal assays are not available (Gombe & Ogada, 1976). (KPIC)

Also, in a more recent update no health concerns affecting workers or other people in and around the factory were observed (Wangai, 2002). (KPIC)

The manufacturing plant of MGK has been extracting, refining and formulating with Pyrethrins for more than 70 years. According to historical data of MGK`s employees, no serious adverse reaction to Pyrethrins have been reported. A few minor cases of dermatitis were noted, however, the implication of Pyrethrins is not confirmed.

The manufacturing plant of BRA in Tasmania has been harvesting and extracting Pyrethrins for many years. Over the last 25 years of manufacturing, no serious health problems have been recorded in workers. Minor skin irritations have been observed following the harvesting operation, however these effects are more likely to be due to the dustiness of the harvested crop. These skin irritations disappeared after washing with clean water and at worst within 1-2 days. Once the harvested crop material has been extracted, no cases of any health issues from handling the extracted and refined products have been noted. (BRA, MGK and SCJ)

Direct observations on clinical cases or poisoning incidents

Based on more than 80.000 calls, the Report to the American Association of Poison Control Centers concludes "that products containing Pyrethrins or pyrethroids can be used with the expectation of no undue risk" (Anonymous, 2001). (KPIC)

Occupational exposure in a Pyrethrum extract factory did not result in clinical symptoms even upon high exposure levels with the exception of minor pleural lesions of the inflammatory type that were attributed to the mechanical irritation of the high levels of dust and were not impairing the health of the individuals affected. (KPIC)

Pyrethrins have been on the market for many years and their applications/uses are numerous. Few isolated incidents have been reported in the literature. In any case, the data provided is not sufficient to establish a relationship between Pyrethrins and the effects and/or symptoms caused. Pyrethrins are not known to cause any serious poisoning incidents, the vast majority of exposures are relatively harmless and self-limited. The most common symptoms include: dermatitis with papules in moist areas, intense pruritus, nausea, a stinging sensation in the nasal and upper pharyngeal mucosa, moderate shortness of breath, a cough productive of white phlegm without haemoptysis, fatigue, headache, dizziness and sensitisation (Garcia-Bravo *et al*., 1995; Paton & Walker, 1988; Wax & Hoffman, 1994). (BRA, MGK and SCJ)

Health records from industry or other sources

No health records have been reported from industry or any other sources. (BRA, MGK and SCJ)

Epidemiological studies on the general population

Analysis of exposures of insecticides containing Pyrethrins and/or Pyrethroids from 1994 to 1999 in the USA to humans was entered into the TESS© database. 81838 people were exposed by a variety of routes to consumer products. 41080 cases were reported to be related to products containing Pyrethrins or Pyrethroids.

One-third of the exposures were through ingestion, while inhalation, dermal and ocular exposures occurred in 27.8%, 26.2% and 10.7% of the cases, respectively. Exposure was unintended in 93.1% of cases, in or around the home in 93% and at work in 5% of cases. Nearly all cases (95%) involve an acute exposure (including continuous or repeated exposure lasting less than 8 hours) to Pyrethrin/Pyrethroid. The most frequently reported symptoms were ocular (22.8%), gastrointestinal (22.3%), dermal (21%), respiratory (12.8%), miscellaneous symptoms (10.1%) and neurologic (9.7%). Children under 5 years were most likely to report ocular symptoms; children between the age of 5 and 9 were more likely to exhibit ocular and dermal symptoms. Adults were diagnosed with a variety of symptoms such as gastrointestinal (34.8%), dermal (33.3%), ocular (27.8%) and respiratory (25.1%). During the investigation, no deaths were registered as being due to Pyrethrins/Pyrethroids. The TESS report revealed a number of limitation of which the most significant is the non-distinction between Pyrethrins and Pyrethroids (Pegus, 2001). (BRA, MGK and SCJ)

Diagnosis of poisoning including specific signs of poisoning and clinical tests

Symptoms include dermatitis with papules in moist areas, intense pruritus, bullae, nausea, moderated shortness of breath and chest tightness, a cough productive of white phlegm, fatigue, headache and dizziness.

Contact dermatitis is the most common effect (HSDB database, 2001). It causes a mild erythematous vesicular dermatitis with papules in moist area and intense pruritus. In some cases, blisters appear. Oedema and skin cracking develop in severe cases. The clinical manifestations of inhalation exposure to Pyrethrins can be local or systemic. Localised reactions confined to the upper respiratory tract include rhinitis, sneezing, scratchy throat, oral mucosal oedema, and even laryngeal mucosal oedema. Localised

reactions of the lower respiratory tract include coughing, shortness of breath and chest pain (Paton et al, 1988). An asthma like reaction occurs with acute exposures in sensitised patients. Allergic rhinitis and pneumonitis were also noted.

Pyrethrins also trigger conjunctival oedema and hyperaemia as if can be irritating to eyes and mucous membrane (HSDB database, 2001). (BRA, MGK and SCJ)

Sensitisation/allergenicity observations

Pyrethrum sensitivity has been reported in a number of cases via different forms. Contact dermatitis is the most common allergic reaction. In few cases, bullae appear. Oedema and cracking develop in severe cases. Local anaphylaxis was noted and described by dermatitis and sudden swelling of face.

Anaphylactic reactions are usually characterised by pallor, tachycardia, diaphoresis. A 43-year-old woman with a history of asthma and ragweed allergy, had an anaphylactic shock following the use of a shampoo containing Pyrethrins for the treatment of head lice (HSDB, 2001). About 50% of persons sensitive to ragweed exhibit cross-sensitivity to Pyrethrum. (BRA, MGK and SCJ)

Specific Treatment in Case of an Accident or Poisoning: First Aid Measure, antidotes and Medical Treatment, if Known

There is no specific antidote for Pyrethrin poisoning. Treatment is symptomatic and supportive. Pulmonary sequelae are treated symptomatically with airway maintenance, oxygen by mask at 10 to 15L/min, and ventilatory assistance as dictated by patient status. Circulatory support may require intravenous fluids and rarely, pressor agents. Pharmacologic treatment of bronchospasm and anaphylaxis uses the standard drugs and management protocols. Bronchospasms are treated with inhaled β2-agonist and oral or parenteral corticosteroids. To assist eye irritation, prescription of proparacaine hydrochloride is recommended.

Vitamin E topical application (Vitamin E oil i.e., dl-α-tocopheryl acetate) is highly effective in relieving paraesthesia. Antihistamines are effective in controlling most allergic reactions. Severe asthmatic reactions, particularly in predisposed persons, may require administration of inhaled β2-agonist and/or systemic corticosteroids. Inhalation exposure should be carefully avoided in the future. Anaphylaxis-type reactions may require subcutaneous epinephrine and respiratory support. Contact dermatitis may be treated by applications of topical corticosteroid preparations. (BRA, MGK and SCJ)

Prognosis following poisoning

Two cases found in the literature were described. After the use of a pesticides containing Pyrethrum extract, a 41-year-old farmer developed an erythematous papular lesion of the hands. The farmer gave up the use of both pesticides, and two years later remained free of dermatitis (Garcia-Bravo *et al*., 1995). Following the application of a flea-killer containing Pyrethrum extract, a young man presented symptoms of shortness of breath, a cough productive of white phlegm and nausea. After 4 hours of emergency department treatment and observation, the man had recovered (Paton & Walker, 1988).

When an allergic reaction was established, future contact with the substance should be avoided. (BRA, MGK and SCJ)

Table A.60 Summary table of further human data

No data are available.

A.2.16. Other data

No data are available.

A.3. Environmental effects assessment

A.3.1. Fate and distribution in the environment

The *Chrysanthemum cinerariaefolium* extract from HCS contains Pyrethrins among other constituents, which may be divided into the two groups Pyrethrins I (consisting of pyrethrin 1, cinerin 1, and jasmolin 1) and Pyrethrins II (consisting of pyrethrin 2, cinerin 2 and jasmolin 2). Among these compounds, Pyrethrin 1 was selected as a surrogate for all the pyrethrins to generate the environmental fate data since it is representative of all other components, it is the component with the highest concentration (53%) and because it is difficult to evaluate the environmental fate properties of a mixture. The ecotoxicological studies were performed with the whole extract and the results based on nominal or measured concentrations of total pyrethrins (the six components in Pyrethrin I and II).

A.3.1.1. *Degradation*

A3.1.1.1 Abiotic degradation

Hydrolysis

The results presented in Table A.61 show that the half-lives for Pyrethrin 1 at pH 5 and pH 7 were 687 and 527 days respectively (Selim S., 1995), only the half-life of 17 days, for the pH 9 buffer system, was considered to be meaningful because of the greatest amount of degradation observed over the 30 day test period. No degradation products > 10% of the applied dose were observed in the pH 5 and pH 7 samples. Therefore, ^{14}C-Pyrethrin 1 was found to be stable in buffers at pH 5 and 7, over the 30-day study period. A single major degradation product (>10% dose) was observed in the pH 9 sample; it was designated as degradate A. Degradate A was identified as ^{14}C-Chrysanthemic acid, produced by hydrolysis of the ester linkage of ^{14}C-Pyrethrin 1. Its concentration at pH 9 increased in proportion to the decrease of Pyrethrin 1, accounting for 61% of the applied radioactivity (AR) after 30 days. The degradation reactions were given by pseudo-first-order kinetics.

Another study was presented (Perboni A., 2015) to assess abiotic hydrolytic transformations of the 6 Pyrethrum extract components (Pyrethrin 1, Cinerin 1, Jasmolin 1, Pyrethrin 2, Cinerin 2 and Jasmolin 2) in an aquatic system at pH values normally found in the environment (pH 4-9) under sterile conditions in the absence of light, according to OECD 111. At Tier 1, all six Pyrethrum extract components showed significant hydrolysis (i.e. 10% hydrolysis was observed after 5 days at 50 °C), apart from Pyrethrin 2 and Cinerin 2, both at pH 4. However, in order to describe hydrolysis of all components in a similar manner and facilitate the comparability of results, Tier 2 was performed with all six components. In the higher tier test, the hydrolysis of the test substances was monitored at pH 4.0 pH 7.0 and pH 9.0 and at 3 different temperatures: 15°C, 25°C and 45°C in the dark for a period of 30 days. Hydrolysis of Pyrethrum extract components was shown to be mainly driven by the pH, but also dependent on the temperatue regime:

- At pH 4 significant but slow hydrolysis was only observed for some components.
- At pH 7 relevant hydrolysis was determined for all components, but generally only at elevated temperatures.
- At pH 9 significant hydrolysis was shown for all six components at all temperatures, resulting in fastest hydrolysis at 45 °C.

The DT50 values for hydrolysis was obtained considering FOCUS kinetics guidance, and SFO (single first order) turned out to be the kinetic model describing best the decrease of concentrations.

For risk assessment purposes in freshwater, the half-life of 115 d at pH 7 and 25ºC for Pyrethrin 1 should be used.

For classification and labelling according to the CLP Guidance, data on hydrolysis e.g. OECD Test Guideline 111 might be considered for classification purposes only when the longest half-life t½ determined within the pH range 4-9 is shorter than 16 days. Based on the above results, hydrolysis data for *Chrysanthemum cinerariaefolium* extract from HCS cannot be considered for classification purposes, since the longest half-life determined within the pH range 4-9 is longer than 16 days.

Table A.61 Summary table - Hydrolysis

Summary table - Hydrolysis							
Method, Guideline, GLP status, Reliability, Key/supportive study	pH	Temp. [°C]	Initial TS concentration, C0[mol/l]	Half-life, DT_{50} [d]	Coefficient of correlation, r^2	Remarks	Reference
US-EPA Pesticide Assessment Guidelines, subdivision N, Series 161-1 GLP Reliability 2	5 7 9 7	25 12	14C-pyrethrin 1 (Batch number CFQ.7422; Purity 98.1%) pH 5: 0.31 to 0.38 mg ^{14}C-pyrethrin 1/L pH 7: 0.35 to 0.38 mg ^{14}C-pyrethrin 1/L pH 9: 0.35 to 0.38 mg ^{14}C-pyrethrin 1/L	687 527 17 1476	0.192 0.331 0.947	-	Selim S. (1995) IIIA-7.1.1.1.1 (BRA, MGK and SCJ) Doc III A7.1.1.1.1 (KPIC)
OECD Guideline 111 (Hydrolysis as a Function of pH) GLP Reliability 1 Key	4, 7, 9	15°C; 25°C and 45°C	Pyrethrin 1 Lot/Batch: XX-82-P1 Purity:99.4% Pyrethrin 2 Lot/Batch: XX-82-P2 Purity: 99.3% Cinerin 1 Lot/Batch: XX-82-C1 Purity: 97.5% Cinerin 2 Lot/Batch: XX-82-C2 Purity: 99.3% Jasmolin 1 Lot/Batch: XX-82-J1 Purity: 99.3%	DT_{50} ranged from 0.4 to 211.6 days DT_{50} for Pyrethrin 1 at 25°C is 115 d at pH 7. At 25ºC and pH 9 values for DT_{50} ranged from 4.2 to 14.9 days.	-	DT_{50} values were calculated for Pyrethrum extract components showing significant hydrolysis. At pH 4 significant hydrolysis was only observed for Cinerin 2 at 45°C, for Jasmolin 1 at all temperatures and for Jasmolin 2 at 25°C and 45°C. At pH 7 significant hydrolysis was observed for all components at 45°C. At 25°C Pyrethrin 1, Cinerin 1, Jasmolin 1 and Jasmolin 2	Perboni, A. (2015) IUCLID 10.1.1.1.a (BRA, MGK, SCJ and KPIC)

			Jasmolin 2 Lot/Batch: XX-82-J2 Purity: 96.6% 0.02 mg/L for each analyte			exhibited significant hydrolysis. At 15°C only Jasmolin 1 was not hydrolytically stable. At pH 9 all components were significantly hydrolysed, whereas at 25°C and 45°C fastest hydrolysis was measured for all components.	

Value used in Risk Assessment	
Value/conclusion	DT_{50} for Pyrethrin 1 at 25ºC is 115 d at pH 7.
Justification for the value/conclusion	Hydrolysis is not considered a relevant degradation pathway for Pyrethrins under environmental relevant conditions.

Phototransformation in water

A photolysis test with *Trans*-[cyclopropane-1-^{14}C] Pyrethrin 1 was performed according to the US EPA Pesticide Assessment Guidelines, Subdivision N, 161-2 (comparable to the test OCDE guideline nº 316 "Phototransformation of chemicals in water - direct photolysis"). To determine the extent and rate of aqueous photolysis of ^{14}C-Pyrethrin 1, test samples, containing nominal concentrations of Pyrethrin 1 ranging from 0.32 to 0.38 mg/L in an HEPES buffer at pH 7, were exposed to natural sunlight and maintained at a mean temperature of 25 ± 1°C for 72 hours. Confirmation of the identity and amount of ^{14}C-Pyrethrin 1 was performed by HPLC with radioisotope detection. The isolated degradate E was analysed by GC/MS. Samples radioactivity was determined by LSC.

Results showed that the photolysis rate of Pyrethrin 1 and the degradate E (isomer of Pyrethrin 1), as principal degradation product (concentration greater than 10% of the initial measured dose -IMD), is consistent with first order kinetic with a half-life of 11.8 hours of sunlight and a coefficient of correlation of 0.99.

Table A.62 Summary table – Photolysis in water

Summary table – Photolysis in water								
Method, Guideline, GLP status, Reliability, Key/supportive study	Initial molar TS concentration	Total recovery of test substance [% of appl. AS]	Photolysis rate constant (kcp)	Direct photolysis sunlight rate constant (kpE)	Reaction quantum yield (φcE)	Half-life (t1/2E)	Remarks	Reference
US-EPA Pesticide Assessment Guidelines, Subdivision N, Series 161-2 GLP Reliability 1 Key	0.32 mg/L to 0.38 mg pyrethrin 1 /L	Radiolabelled pyrethrin 1, product code CFQ.7422, Purity 98.1% Non-radiolabelled pyrethrin 1, product code NK9212, Purity 97% Buffer solution (pH 7): mean overall recovery = 97.4% (range: 91.3 to 102.5%)	Not stated	0.059 h^{-1}	1.2 x 10^{-3} in water at pH 7	11.8 h (^{14}C-Pyrethrin 1 +E-isomer)		Selim, S. (1995) and Werle, H. (1991) Doc III IIIA-7.1.1.1.2 (KPIC, BRA, MGK and SCJ)

Conclusion
There are two phases in photodegradation pattern of ^{14}C-Pyrethrin 1.^{14}C-Pyrethrin 1 is rapidly equilibrates with its 'E' isomer when they are exposed to natural sunlight in an aqueous solution buffered to a pH 7, followed by photolysis of both compounds forming numerous minor photolytic products. Therefore, ^{14}C -Pyrethrin 1 is rapidly photolysed when is exposed to natural sunlight in a pH 7-buffered solution, with a half-life of 11.8 hours.

Value used in Risk Assessment	
Value/conclusion	Photolytic half-life in water estimated to be 11.8 h.
Justification for the value/conclusion	Refer to page above.

Estimated photo-oxidation in air

Following the physical and chemical properties and the structure of Pyrethrins it is assumed that degradation and persistence of the active substance mainly depends on reaction with hydroxyl-radicals and the average concentration of hydroxyl-radicals in air. Total OH rate constant was determined to be 281.1508 x 10^{-12} cm³/molec.*sec., mainly due to addition to olefinic bonds (96%) and hydrogen abstraction (4%). Other mechanisms do not contribute to hydroxyl radical estimations. The total rate of both, OH and ozone constant is very low. Half-life in the troposphere was calculated to be 27.391 min for overall OH rate constant and 29.562 min for ozone rate constant. Following the Atkinson calculation, the chemical half-life for Pyrethrum in the troposphere will be below 1 h. It is therefore concluded that Pyrethrins will not accumulate in air and will only be transported on very short distances.

The photochemical oxidative degradation half-life of Pyrethrin 1 in air was calculated according to the method developed by Atkinson[25], which is based on the structural activity relationship (QSAR´s), by using the Atmospheric Oxidation Program v 1.91 (AOPWIN-software). These estimations were carried out with respect to the OH radical and ozone reactions, using a 12-hours-day with 300.95 x 10^{-12} and 96.32 x 10^{-17} cm^3/molecule-sec, respectively. The half-lives for the hydroxyl and ozone reactions in air are estimated to be 25.59 and 17.13 minutes, respectively.

A half-life of 76.8 minutes for Pyrethrin 1 in air has been estimated using a 24-hour-day and assuming an OH radical concentration of 5 x 10^5 radicals.cm^{-3} according to AOPWIN version 1.91 and following recommendations of ECHA Guidance on Risk Assessment, Chapter 2.3.6.3[26].

Table A.63 Summary table – Photo-oxidation in air

Summary table – Photo-oxidation in air					
Model	Light protection (yes/no)	Estimated daily (24h) OH concentration [OH/cm³]	Overall OH rate constant [cm³/molecule/sec]	Half-life [min]	Reference
No Guideline available		1.5 x 10^6	300.95 x 10^{-12}	25.59	O'Carroll, N. (2005) IIIA-7.3.1 (BRA, MGK and SCJ)

[25] Atkinson, R. (1988). Estimation of Gas-Phase Hydroxyl Radical Rate Constants for Organics Chemicals. Environ. Toxicol. Chem. 7:435-442.
[26] Guidance on BPR: Vol IV Environment Parts B+C, Version 2.0 October 2017

Estimation method by AOPWIN version 1.91		5 x 10^5		76.8	IIIA-7.3.1 (BRA, MGK and SCJ)
Calculation			281.1508 x 10^{-12}	<1 hour	Oellrich (2000), Doc III A7.3.1 (KPIC)
No Guideline available		7 x 10^{11} (ozone concentration)	96.32 x 10^{-17} (Ozone reaction rate constant)	17.13	IIIA-7.3.1 (BRA, MGK and SCJ)

Value used in Risk Assessment	
Value/conclusion	Photo-oxidation in air DT_{50} = 76.8 min
Justification for the value/conclusion	Considering all the available information, Pyrethrin 1 is not expected to volatilise in air in significant quantities. The small amounts that may volatilise are rapidly degraded via reaction with OH radicals and ozone. Based on the short half-life of Pyrethrin 1 in the atmosphere , accumulation and contamination by wet or dry deposition in the atmosphere are not to be expected. Therefore, air will not be an environmental compartment of concern for Pyrethrin 1 used as insecticide for household.

Photolysis on soil

^{14}C-pyrethrin 1 was found to rapidly photolyse when exposed to sunlight on the surface of soil to form numerous degradates including CO_2. No single degradate represented more than 10% of the initially applied radioactivity. Within 24 h, a mean of less than 17% of the initial concentration of ^{14}C-pyrethrin 1 remained. Non-exposed control samples had more than 55% of the initial concentration of ^{14}C-pyrethrin 1 remaining at the end of the 24 h period. The photolysis is consistent with first order kinetics with a half-life of 12.9 h. The ^{14}C-pyrethrin 1 in the non-exposed test system also degraded during the 24 h test period, but at a slower rate than the exposed samples. The half-life of the non-exposed samples was determined to be 82.9 h. The results indicate that ^{14}C-pyrethrin 1 in the exposed samples was degraded via both photolytic (fast) and non-photolytic (slower) pathways.

The Spain CA has adjusted values of calculated DT_{50} at 25ºC to the equivalent at 12ºC using equation 28 in the ECHA Guidance[27]. Thus, the DT_{50} of Pyrethrin 1 under the average EU outdoor temperature was calculated in 36.12 hours.

Table A.64 Summary table – Photolysis on soil

Summary table – Photolysis on soil					
Guideline /Test method	Initial molar TS concentration [mg.kg-1]	Temperature [°C]	Total recovery of test substance [% of appl.a.s.]	Half-life (t1/2E) [h]	Reference
USEPA FIFRA N-161-3, 40 CFR Sec.	^{14}C-pyrethrin 1 (Batch number CFQ.7422;	24 ± 2°C	Mean overall recovery = 96.6%	12.9 hours	Testman R. (1994), Doc III A7.2.2.4

[27] Guidance on BPR: Vol IV Environment Parts B+C, Version 2.0 October 2017

158.130 GLP Reliability 1 Key	Purity 95.9%) 9.3 mg pyrethrin 1/L				(KPIC) IIIA- 7.2.2.4 (BRA, MGK and SCJ)

A3.1.1.2 Biotic degradation

A3.1.1.2.1 Biodegradability (ready)

One biodegradation study according to OECD guideline 301B and the US EPA Method 835.3110 (CO_2 evolution test) is available for the Refined Pyrethrum Extract. The test substance was added to two vessels containing mineral salts medium inoculated with activated sludge at a nominal test concentration of 10 mg Carbon/L. Sodium benzoate was used as reference substance. An additional mixture containing Sodium benzoate and Refined Pyrethrum Extract was established in order to assess the potential of the test substance for microbial inhibition.

The results in Table A.65 show that mean cumulative CO_2 production by mixtures containing Refined Pyrethrum Extract accounted for 46% by the end of the test on day 29. The reference substance, Sodium benzoate, was degraded by 66% after 7 days and 93% after 29 days in the absence of Refined Pyrethrum Extract, and by 69% after 7 days in its presence, which confirmed that Refined Pyrethrum Extract was not inhibitory to the activity of the microbial inoculum. (BRA, MGK and SCJ)

Results of the standard test indicate that Pyrethrum extract is neither readily nor inherently biodegradable. However, since Pyrethrum extract shows a low solubility in water, the lack of microbial degradation could be a consequence of the limited availability of Pyrethrum extract for micro-organisms.

In addition another test was performed according to OECD 301 (Koopmans 1995). Pyrethrum Extract was added to two vessels containing mineral salts medium inoculated with activated sludge at a nominal test concentration of 12 mg TOC/L. The relative degradation values calculated from the measurements performed during the test period revealed no significant (>10%) degradation of Pyrethrum Extract (see Table below).

Table A.65 Summary table - biodegradation studies (ready/inherent)

Summary table - biodegradation studies (ready/inherent)											
Method, Guideline, GLP status, Reliability, Key/supportive study	Test type	Test parameter	Inoculum			Additional substrate	Test substance concentr.	Degradation		Remarks [positive control]	Reference
			Type	Concen-tration	Adap-tation			Incubation period	Degree [%]		
OECD 301 B GLP Reliability 2 Key	Ready Biodegradability	CO_2 evolution test	Activated sludge	Refined Pyrethrum Extract (Batch number FEK-99; purity 57.03%) 10 mL sludge/L mineral medium	No	No	10 mg/L	29 days	46	-	Barnes, S. (2002) IIIA-7.1.1.2.1 (MGK, BRA and SCJ)
OECD 301 B GLP Reliability 2 Key	Ready Biodegradability	CO_2 evolution test	Activated sludge	Pyrethrum Extract (Batch 94/10.7; Purity 25.14% total Pyrethrins) 10 mL sludge/L mineral medium	No	No	15 mg Pyrethrum Extract /L	28 days	4.6%	-	Koopmans (1995), Doc III A7.1.1.2.1 (KPIC)

As the pass level (carbon dioxide production equal to or greater than 60% of the theoretical value) within the 28 days incubation period in this test on ready biodegradability is not fulfilled by Refined Pyrethrum Extract, representing *Chrysanthemum cinerariaefolium* extract from HCS, so the a.s. cannot be classified as readily biodegradable.

Value used in Risk Assessment	
Value/conclusion	Not readily biodegradable
Justification for the value/conclusion	See above

A3.1.1.3 Rate and route of degradation including identification of metabolites and degradation products

A3.1.1.3.1 Biological sewage treatment

Aerobic biodegradation

No data are available.

Anaerobic biodegradation

No data are available.

STP simulation test

No data are available.

A3.1.1.3.2 Biodegradation in freshwater

Aerobic aquatic degradation

The objective of the study from Hein and Moendel (2017) was to provide information on the degradation rate (DT 50, DT 90) and metabolism of Pyrethrin 1 in natural water. Additionally, information on the identity and quantity of transformation products in water including a mass balance was determined. Test systems containing natural water were treated using [cyclopentenone-2-^{14}C] Pyrethrin 1, at two different nominal concentrations 10 µg ("low dose") Pyrethrin 1/L and 100 µg ("high dose") Pyrethrin 1/L. In the test, calculated SFO DT50 values for pyrethrin 1 ranged from 6.7-10.7 days (at 20 ± 2°C). The main degradation product was pyrethrolone which reached a maximum of 9.5% AR after 21 days and then decreased to 2.8% AR at the last sampling interval. Several non-identified fractions were detected but these were minor and/or composed of several peaks. Mineralisation reached a maximun of 7% by the end of the study.

Table A.66 Summary table – freshwater aerobic biodegradation

Summary table – freshwater aerobic biodegradation							
Method, Guideline, GLP status, Reliability, Key/supportive study	Test type	Exposure	Test substance concentration	Incubation period	Degradation (DT_{50})	Remarks	Reference
OECD Guideline for Testing of Chemicals, No 309 "Aerobic Mineralisation in Surface Water", Apr. 13, 2004 GLP Reliable 1	OECD	darkness under aerobic conditions in the laboratory at 20 ± 2°C	[cyclopentenone-2-^{14}C] pyrethrin 1, (Batch CFQ42752; Purity >97.7%) 10 µg/L (i.e. "low dose") and 100 µg/L (i.e. "high dose")	62 days	Calculated SFO DT_{50} values ranged from 6.7-10.7 days	The major degradation product was pyrethrolone which reached a maximum of 9.5% AR after 21 days and then decreased to 2.8% AR at the last sampling interval.	Hein W. *et al.*, 2017, IUCLID 10.1.3.2 Doc IIIA / Section 7.1.2.1.1.1

Value used in Risk Assessment	
Value/conclusion	DT_{50} water 20ºC (d) 10.7
Justification for the value/conclusion	Using SFO kinetics DT_{50} values ranging from 6.7-10.7 days were obtained (Hein *et al*., 2017). The highest DT_{50} was used as a worst-case.

Water/sediment degradation test

Pyrethrin 1 degraded at a very rapid rate when applied to an aerobic aquatic environment (1 ppm dosing) (Robinson and Wisocky, 1994). Degradation proceeded initially by oxidation to form chrysanthemic acid and a number of low level degradants. Residues in water and sediment were initially extracted but extended degradation was accompanied by the formation of residues that were bound to sediment humus fractions and appeared to be partially comprised of bound chrysanthemic acid. Mineralisation was an observed but minor degradation pathway (4% CO_2 at day 30). The principal extractives were apart from pyrethrin 1, chrysanthemic acid, the major metabolite, and three additional minor degradates. A clear pattern of build-up and decline emerged for chrysanthemic acid, resulting in a maximum occurrence in the water/sediment system of 21.2% of applied radioactivity on day 21. The minor degradates were detected at various intervals, none exceeding 5% of the initial concentration of pyrethrin 1 at any point. The half-life of the pyrethrin 1 in the water/sediment system tested was calculated to be 10.5 days following pseudo-first-order kinetics at 25 ºC. This is equivalent to 29.7 days at 12 ºC. Later the applicant recalculated endpoints based on FOCUS Guidance as shown below.

In this test, however, only one sediment was used for determination of degradation rates whereas Guidance indicates that at least two sediments and their associated water should be used for calculations. In addition, the test finished before guidance indicates, at day 30, when still 14.7 % of the substance was present.

In a second study conducted by Witte (2007), pyrethrin 1 was observed to degrade rapidly when applied to two separate water/sediment systems taken from the natural environment. This occurred via a rapid movement from the water phase into the sediment phase combined with a steadily increasing mineralization to CO_2 (30 – 51% at test end) and breakdown in both aquatic and sediment phases to the metabolite chrysanthemic acid (maximum 65.6 and 66.8%). A clear pattern of build-up and decline emerged for Chrysanthemic acid in the water and sediment phases of both systems. A substantial portion of the applied radioactivity (30 – 40% at test end) became bound to sediment in both test systems. Following first order kinetics, the half-lives for Pyrethrin 1 and chrysanthemic acid in the whole water/sediment system ranged from 1.6 to 2.4 days and 18 to 109 days, respectively.

The anaerobic metabolism of ^{14}C-Pyrethrin was studied under laboratory conditions in a water sediment model system at an initial concentration of 10 mg/L at 25ºC (Robinson and Wisocky 1995). With the extraction method used, 46.19% of the AR remained unextractable from sediment after 1 year. Three main extractable degradation products were found: Cyclopropane diacid (a maximum of 14.6% at 364 days), ^{14}C-Chrysanthemic acid (a maximum of 12.6% at 31 days) and a reduced form of Pyrethrin 1 named Jasmolin 1 (a maximum of 10.0% at 180 days). Sediment fulvic fractions-bound residues were also found to contain ^{14}C-Chrysanthemic acid as a terminal residue. All other degradation products were found at concentrations below or equal to 4% of the AR. In this system volatiles were formed in low amounts of 13.92% of the AR at day 365 (4% of the AR at day 30), so mineralization process was low. Therefore, mechanisms involved in degradation of 14C-Pyrethrin appear to be a combination of reductive and oxidative process.

^{14}C-Pyrethrin dissipated under anaerobic aquatic sediment conditions with a calculated half-life of 86 days for the whole system (240.8 days reflecting the average EU outdoor temperature of 12ºC).

Table A.67 Summary table – fresh water/sediment degradation

Summary table – fresh water/sediment degradation								
Method, Guideline, GLP status, Reliability, Key/supportive study	**Exposure**	**Test system**		**Test substance concentration**	**Incubation period**	**Degradation (DT_{50})**	**Remarks**	Reference
		Water	Sediment					
US EPA Subdiv. N, § 162-4 Chemistry: Environmental Fate GLP Reliability 2 Key	25 ±1°C	Pond	Pond in Lucama, North Carolina, USA	^{14}C-pyrethrin 1 (Batch number CFQ 7390; Purity 98.7% - 99.7%) ca. 10 ppm	30 days	10.5 days	pseudo-first-order kinetics	Robinson, R. A.; Wisocky, M.J. (1994), Doc III A7.1.2.2.2 (KPIC) (MGK, BRA and SCJ)
OECD 308 (April 2002), SETAC 1995 GLP Reliability 2 Key	20 ±2°C	Pond	Ensingen, district of Enz, Germany Sandy silt	56.0 µg ^{14}C-pyrethrin 1 (56.6 µg unlabelled pyrethrin 1) [cyclopropane-1-^{14}C] pyrethrin 1 (Lot No. CFQ14811 Batch 1); Purity Radiochemical 98.4%) Pyrethrum Pale Extract (Batch number 2006/3-3/Pale; Purity Pyrethrins I: 30.65% w/w Pyrethrins II: 19.49% w/w Total Pyrethrins: 50.14% w/w)	-	1.6 days whole system	-	Witte A. (2007), Doc III A7.1.2.2.2/02 (KPIC)
	20 ±2°C	Creek	Spiegelberg, district of Rems-Murr, Germany Sand		-	2.4 days whole system	-	

Summary table – fresh water/sediment degradation								
Method, Guideline, GLP	**Exposure**	**Test system**		**Test substance concentration**	**Incubation period**	**Degradation (DT_{50})**	**Remarks**	Reference
US EPA Pesticide Assessment Guidelines, Subdivision N, Series 162 – 3 GLP Reliability 2	Anaerobic at 25ºC in the dark Anaerobic at 12ºC in the dark	24.28% (supernatant)	Sandy loam 1.3% Organic carbon pH=4.7 16.28% (extractable residues) 46.19 (PES)	*Trans*-[cyclopropane-1-^{14}C] Pyrethrin 1 (Batch NB8309; > 97.99% (radiochemical purity)) 10 mg/L	364	86.1	-	Robinson, R.A. and Wisocky, M.J. (1995) IIIA-7.1.2.2.2 (BRA, MGK and SCJ)
1 Test according to OECD criteria								

Conclusion

Investigations of degradation and metabolism behaviour of ^{14}C-Pyrethrin 1 in water-sediment systems were conducted under aerobic and anaerobic conditions in compliance with US EPA Pesticide Assessment Guidelines, Subdivision N, Series 162 – 4 and 3, respectively (comparable to OECD method 308). Pyrethrins 1 was observed to degrade fast under aerobic conditions, with DT_{50} value of 10.5 days. Pyrethrin 1 was observed to degrade more slowly under anaerobic conditions, with DT50 value of 86 days. A common metabolite was identified in both systems Chrysanthemic acid which can be partially bioavailable for aquatic and sediment organisms. Mineralization of Pyrethrin 1 reached a maximum of 50% in the study by Witte 2007. Considering the anaerobic degradation there is an important percentage of bound residues (non-extractable residues) in the sediment.

According to the CLP Guidance a substance is not rapidly degradable unless it is demonstrated to be primarily degraded biotically or abiotically e.g. via hydrolysis, in the aquatic environment with a half-life <16 days (corresponding to a degradation of > 70 % within 28 days), and it can be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment. *Chrysanthemum cinerariaefolium* extract from HCS does not meet these requirements.

The DT_{50} values of the study by Robinson and Wisocky (1994) were recalculated based on the FOCUS guidance.

Please find below details of the $DT_{50\ system}$ values for pyrethrin 1 used for PEC calculations for *Chrysanthemum cinerariaefolium* extract from HCS.

Water / sediment system	**pH water phase**	**pH sed**	**t °C**	**DT50/ DT90 system (d)**	**St. (r^2)**	**DT50/ DT90 water (d)**	**St. (r^2)**	**DT50/ DT90 sed (d)**	**St. (r^2)**	**Method of calculation**
Sandy loam*	6.8	4.7	25 20	7.93/26.4 11.83/39.38	χ^2=7.3	n.c.	-	n.c.	-	SFO
Sandy silt**	8.34	7.2	20	5.26/17.55	0.947	1.3/4.3	0.986	n.c.	-	SFO
Sand**	8.24	7.4	20	2.36/7.85	0.870	0.7/2.3	0.867	n.c.	-	SFO
Geometric mean ($DT_{50system}$ 20°C):				**5.27**						
DT50 12ºC				**11.2**						

* = Robinson and Wisocky (1994)

** = Witte (2007)

n.c. = not calculated

SFO = Single First Order kinetic

Value used in Risk Assessment	
Value/conclusion	DT_{50} for biodegradation in water/sediment 11.2 d (at 12ºC) (Pyrethrin 1)
Justification for the value/conclusion	5.27 d at 20ºC

A4.1.1.3.3 Biodegradation in seawater

Seawater degradation study

No data available.

Seawater/sediment degradation study

No data available.

A4.1.1.3.4 Higher tier degradation studies in water or sediment

No data available.

A4.1.1.3.5 Biodegradation during manure storage

No data available.

A4.1.1.3.6 Biotic degradation in soil

No data available.

A4.1.1.3.7 Laboratory soil degradation studies

Aerobic biodegradation

Pyrethrin 1 degraded in soil under aerobic conditions following treatment of a sandy loam soil at approximately 1 ppm (Robinson, 1994). Degradation occurred with the rapid loss of extractable soil residues, the formation of CO_2 (43.41% by day 181) and soil bound residues. Total pyrethrin 1 content in organic extracts declined from an average level of 85.42% of the applied radioactivity on day 0 to less than 1% by day 59 after application. Degradation proceeded initially by a combination of hydrolysis and oxidation to form a number of low level metabolites. Residues in soil were initially extracted, but extended degradation was accompanied by the formation of residues that were bound to soil humus fractions and ultimately mineralized, 43.41% CO2 at the end of the test. In addition to the evolution to CO2, four main metabolites (A to D) were found along with four metabolite regions where no single metabolite could be defined. None of the metabolites identified reached a concentration higher than 5%.

The objective of the study by Hein W. (2017) was to determine the degradation route and rate of [Cyclopentenone-2- ^{14}C]Pyrethrin 1 under aerobic conditions in soil at 20 °C. The concentration of Pyrethrin 1 and possible metabolites was determined throughout the test duration only in soil (loam), including the formation of volatile products such as carbon dioxide generated by mineralization. Material balances were established at each sampling interval which showed the distribution of Pyrethrin 1 and any formed metabolites as a function of time. In the test, Pyrethrin 1 decreased continuously during the course of the study. Pyrethrin 1 was degraded mainly by mineralisation (51.07 % (mean) of the AR at day 120) and formation of bound residues (33.51 % (mean) of the AR at day 120). No other significant metabolites (>5 %) were detected.

For comparative purposes, additional studies (Fifi 2015 a, b and c) were conducted to determine the rate of degradation of the six components of Pyrethrum extract (Pyrethrin 1, Cinerin 1, Jasmolin 1, Pyrethrin 2, Cinerin 2 and Jasmolin 2) in 3 soils under aerobic conditions at 20°C in the dark. All six pyrethrin esters exhibit very low persistence with DT50 values ranging from 0.4 (pyrethrin 2) to 10.7 days (jasmolin 1), calculated according to FOCUS 2006 as far as possible. Based on these data it is concluded that pyrethrin 1 is representative of the pyrethrins.

The soil metabolites analysed were not found in relevant amounts throughout the incubation period. Soil concentrations of Chrysanthemic acid, Pyrethric acid, Pyrethrolone, Cinerolone and Jasmolone were always below the LOQ. In addition Pyrethric acid, Cinerolone and Jasmolone did not exceed the LOD.

The half-life of pyrethrin 1 was calculated to be 3.3 days (pseudo-first-order kinetics). Four main metabolites (A to D) were designated. Metabolite D was identified as chrysanthemic acid. None of these metabolites amounted at any time to more than 10% of the initial pyrethrin 1 concentration.

Table A.68 Summary table – aerobic biodegradation in soil- laboratory study

Method, Guideline, GLP status, Reliability, Key/supportive study	**Exposure**	**Test system** Soil origin	Soil type	pH	OC%	**Test sub-stance concentration**	**Incubation period**	**Degrad-ation DT_{50}**	**Remarks**	**Reference**
US EPA Pesticide Assessment Guidelines, Subdivision N, Series 162 – 1 GLP Reliability 2 Key	Aerobic at 25 ºC in the dark 12 ºC 10 ºC	Grand Forks County, North Dakota, USA	Sandy loam	6.4	2.2%	1 mg/kg (^{14}C-pyrethrin 1 (Batch number CFQ 7390; Purity 96.08% - 97.14% (radiochemical purity))	181 days	1.88 d at 25°C 2.47 d at 20°C 4.68 d at 12°C	The DT_{50soil} of 1.88 d (study value at 25°C, recalculated using SFO) normalized to 12°C is: 5.32 d. The DT_{50soil} of 2.47 d (study value of 1.88 d normalized to pF2 and 20°C) normalized to 12°C is: 4.68 d.	Robinson (1994), Doc III A7.2.1 (KPIC) IIIA-7.2.2 (BRA, MGK and SCJ)
OECD: Guideline 307; Aerobic and Anaerobic Transformation in Soil, April 24, 2002 GLP Reliability 1 Key	Aerobic at 25 ºC in the dark, 19.4°C ± 0.7°C and a soil moisture content of about pF 2.5	Mußbach, Germany	Loam	7.28	1.73	0.1 mg/kg dwt [cyclopentenone-2-^{14}C] pyrethrin 1, Batch CFQ42752; Radiochemical purity: >99%	120 days	DT_{50} value was 4.05 days using first order kinetics. The DT50 value was recalculated to 2.96 days at 20 ºC and pF 2.	Pyrethrin 1 was degraded mainly by mineralisation (51.07% (mean) of the AR at day 120) and formation of bound residues (33.51% (mean) of the AR at day 120). No other significant metabolite (>5%) was detected.	Hein W. (2017), IUCLID 10.2.1 Doc IIIA / Section 7.2.1

Method, Guideline, GLP status, Reliability, Key/supportive study	Exposure	Test system				Test sub-stance concentration	Incubation period	Degrad-ation DT_{50}	Remarks	Reference
		Soil origin	Soil type	pH	OC%					
OECD: Guideline 307; Aerobic and Anaerobic Transformation in Soil, April 24, 2002 GLP Reliability 1 Key	aerobic conditions at 20°C and 45% MWHC in darkness	Loam Soil (batch F2.40815), Lufa-Speyer, Lufa code 2.4	Loam	7.30	2.21	0.1 mg/kg dwt pyrethrin extract containing six pyrethrin esters (pyrethrin 1, cinerin 1, jasmolin 1, pyrethrin 2, cinerin 2 and jasmolin 2)	120 days	DT_{50} values 2.1 and 9.0 days. The pseudo SFO DT_{50} value for pyrethrin 1 normalised to pF 2 was 4.69 days.	-	Fifi (2015a), IUCLID 10.2.1 Doc IIIA / Section 7.2.1
OECD: Guideline 307; Aerobic and Anaerobic Transformation in Soil, April 24, 2002 GLP Reliability 1 Key	aerobic conditions at 20°C and 45% MWHC in darkness	Loamy Sand Soil (batch F2.20815), Lufa-Speyer, Lufa code 2.2	Loamy Sand Soil	5.25	1.56	0.1 mg/kg dwt pyrethrin extract containing six pyrethrin esters (pyrethrin 1, cinerin 1, jasmolin 1, pyrethrin 2, cinerin 2 and jasmolin 2)	120 days	DT_{50} values 0.4 days and 5.4 days. The pseudo SFO DT_{50} value for pyrethrin 1 normalised pF2 was 3.9 days.	-	Fifi (2015b), IUCLID 10.2.1 Doc IIIA / Section 7.2.1
OECD: Guideline 307; Aerobic and Anaerobic Transformation in Soil, April 24, 2002 GLP Reliability 1 Key	aerobic conditions at 20°C and 45% MWHC in darkness	Sandy loam soil (batch F5M0815), Lufa-Speyer, Lufa code 5M	Sandy loam soil	7.40	1.00	0.1 mg/kg dwt pyrethrin extract containing six pyrethrin esters (pyrethrin 1, cinerin 1, jasmolin 1, pyrethrin 2, cinerin 2 and jasmolin 2)	120 days	DT_{50} values 0.5 days and 5.3 days. The pseudo SFO DT_{50} value for pyrethrin 1 normalised to 20ºC and pF 2 was 3.0 days.	-	Fifi (2015c), IUCLID 10.2.1 Doc IIIA / Section 7.2.1

In summary, the rate of aerobic degradation of pyrethrin 1 was investigated in 5 soils at 20-25ºC and various moisture contents (45% MWHC, 75% of pF 2.5, pF 2.5) in darkness. Degradation was rapid and results, including normalised values, are shown in the table below.

Table A.69: Laboratory kinetic and statistical analysis of pyrethrin 1

Study	**System**	**Kinetic model**	**pH**	**Mo**	**Parameter (K, K1, k2, g, tb,α, β)**	**χ^2,%-error**	**DT_{50} [days]**	**DT_{90} [days]**	**SFO DT_{50} 20ºC, pF2**
Robinson, 1994	North Dakota (sandy loam)	SFO	6.4	85.17	k = 0.3687	12.9	1.9	6.3	2.5
Hein, 2017	Mußbach (loam)	SFO	7.28	90.2	k = 0.171	6.89	4.1	13.5	2.96
Fifi, 2015a	Loam	FOMC	7.30	-	n/s	6.16	6.6*	21.9	4.69
Fifi, 2015b	Loamy Sand	FOMC	5.25	-	n/s	13.37	3.9*	12.8	3.9
Fifi, 2015c	Sandy loam	SFO	7.40	-	n/s	5.7	3.0	9.9	3.0
Geomean									3.3

* as SFO

Value used in Risk Assessment	
Value/conclusion	DT_{50} for biodegradation in soil 3.3 d (at 20ºC) (Pyrethrin 1)
Justification for the value/conclusion	Refer to the table above.

Anaerobic biodegradation

No data available.

A4.1.1.3.8 Higher tier degradation studies in soil

Field dissipation studies (field studies, two soil types)

Pyrethrum (measured as Pyrethrins 1) applied to bare ground soil in a single application of Pyrenone Crop Spray at the maximum labelled seasonal rate (0.52 kg/ha) exhibited a half-life of approximately 1-2 h. No residues of Pyrethrin 1 above the 0.1 ppm limit of quantitation were detectable in any soil core samples by 1 day after application and beyond. Storage stability data indicated that Pyrethrins 1 residues from all samples should have been stable until analysis. Transit stability data indicated stability of Pyrethrins I in frozen soil. Results from the field trial suggest that residues of Pyrethrum dissipated completely over a 2 day period.

Table A.70 Summary table – Field dissipation

Summary table – Field dissipation										
Method, Guideline, GLP status, Reliability, Key/supportive study	Site	Application rate (g AS/ha)	Surface	Soil type	Soil texture	Test duration	Degradation DT_{50}[a]	Degradation DT_{90}[a]	Remarks	Reference
USEPA Subdivision N, Section 164-1 GLP Reliability 2 Key	California/USA	0.52 kg a.i./ha Pyrenone Crop Spray (Batch number M60008 ; contains 6% Pyrethrum and the synergist Piperonyl Butoxide. Pyrethrum again is composed of Pyrethrins I (pyrethrin 1, cinerin 1, jasmolin 1) and Pyrethrins II (pyrethrin 2, cinerin 2, jasmolin 2))	Bare ground soil	Sandy loam	Sandy loam	179 days	0.04	0.15	k1 (1/day) = 15.475	Hattermann D.R. (1992), Doc III A 7.2.2.2/01&02 (KPIC)
	Georgia/USA			Sandy loam	Sandy loam		0.04	0.12	k1 (1/day) = 19.202	
	Michigan/USA			Sandy loam	Sandy loam		0.08	0.26	k1 (1/day) = 8.988	

[a] degradation values presented as single first order values

Value used in Risk Assessment	
Value/conclusion	DT_{50} for biodegradation in soil 3.3 d (at 20ºC) (Pyrethrin 1)
Justification for the value/conclusion	Refer to Section A4.1.1.3.7

A4.1.1.3.9 Short summary and overall relevance of the provided information on degradation and conclusion on rapid degradation

Hydrolysis

The half-lives for Pyrethrin 1 at pH 5 and pH 7 were 687 and 527 days respectively. At pH 9 the substance half life was 17 days. Temperature of the test was 25ºC. No degradation products > 10% of the applied dose were observed in the pH 5 and pH 7 samples. A single major degradation product, identified as Chrysantemic acid (>10% dose) was observed in the pH 9 sample. Its concentration at pH 9 increased in proportion to the decrease of Pyrethrin 1, accounting for 61% of the applied radioactivity (AR) after 30 days. The degradation reactions were given by pseudo-first-order kinetics.

In another study (Perboni A., 2015) abiotic hydrolytic transformations of the 6 Pyrethrum extract components (Pyrethrin 1, Cinerin 1, Jasmolin 1, Pyrethrin 2, Cinerin 2 and Jasmolin 2) in an aquatic system at pH values normally found in the environment (pH 4-9) under sterile conditions in the absence of light, according to OECD 111. In the higher tier test, the hydrolysis of the test substances was monitored at pH 4.0 pH 7.0 and pH 9.0 and at 3 different temperatures: 15°C, 25°C and 45°C in the dark for a period of 30 days. The half-life of 115 d at pH 7 and 25ºC for Pyrethrin 1 was determined.

Biodegradability (ready)

Results of the standard tests with Pyrethrum Extract indicate that the active substance is not readily degradable. However, since Pyrethrum extract shows a low solubility in water, the lack of microbial degradation could be a consequence of the limited availability of Pyrethrum extract for micro-organisms.

Water and Water/sediment degradation test

In water, calculated SFO DT50 values for pyrethrin 1 ranged from 6.7-10.7 days (at 20 ± 2°C). The main degradation product was pyrethrolone which reached a maximum of 9.5% AR after 21 days and then decreased to 2.8% AR at the last sampling interval. Several non-identified fractions were detected but these were minor and/or composed of several peaks. Mineralisation reached a maximun of 7% by the end of the study.

In aerobic water/sediment systems Pyrethrins 1 was observed to degrade under aerobic conditions, with DT_{50} values ranging from 1.6 to 10.5 days. A substantial portion of the applied radioactivity became bound to sediment in the tests. A common metabolite was identified in both systems: Chrysanthemic acid, which can be partially bioavailable for aquatic and sediment organisms. Mineralization of Pyrethrin 1 reached a maximum of 50% under aerobic conditions.

Aerobic biodegradation in soil

In soil, the half-life of pyrethrin 1 was calculated to be 3.3 days at 20ºC (pseudo-first-order kinetics, geomean of 5 studies). Four main metabolites (A to D) were designated. Metabolite D was identified as chrysanthemic acid. None of these metabolites amounted at any time to more than 10% of the initial pyrethrin 1 concentration. Maximun mineralization reached was 51.03 %.

Conclusion

Chrysanthemum cinerariaefolium extract from HCS is a complex substance of natural origin. According to the Guidance on the Application of the CLP criteria (version 5, July 2017) a complex substance, such as UVCBs, should be regarded as not rapidly degradable if the constituents that are not rapidly degradable constitute a significant part of the substance, e.g. more than 20%, or for a hazardous constituent an even lower content.

Chrysanthemum cinerariaefolium extract from HCS contains fate is represented by Pyrethrin 1. Hydrolysis data for this component yields half-lives > 16 days across different pHs at 25ºC. According to the Guidance on the Application of the CLP criteria (version 5, July 2017), data on hydrolysis might be considered for classification purposes only when the longest half-life determined within the pH range 4-9 is shorter than 16 days. Thus, hydrolysis cannot be considered for classification purposes in this case.

In water tests Pyrethrin 1 was not ultimately degraded, not meeting the guideline requirement of a half-life <16 days (corresponding to a degradation of > 70 % within 28 days).

In water/sediment the substance primary degraded with DT50 values ranging from 1.62 to 10.5 days at tests temperature and transformed into metabolites harzardous to the aquatic environment or non-identified metabolites. A part of the substance also bound to sediment.

Further, ready biodegradation available for Pyrethrum Extract showed that the substance is not ready biodegradable.

Based on the above *Chrysanthemum cinerariaefolium* extract from HCS is considered not rapidly degradable.

A.3.1.2. *Distribution*

A4.1.2.1 Adsorption onto/desorption from soils

The binding potential of radiolabelled pyrethrin 1 was studied in four soils using the batch equilibration method (Reynolds & Robinson, 1994). A 1:100 soil-to-solution ratio was used, and the equilibration time for both adsorption and desorption was three hours. At a rate of 0.75 ppm, 70.86%, 75.22%, 77.30% and 49.43% of total radioactivity adsorbed to solids of sandy loam, silty clay loam, silt loam and sand, respectively. The major radioactive product was the parent compound (pyrethrin 1). Low levels of the chrysanthemic acid, a hydrolysis product, were also detected in organic extracts from the adsorption phase, but none exceeded 5.16% of the applied radioactivity. The adsorption and desorption constants obtained in the study indicated that parent chemical was immobile in all of the examined soils and was not readily desorbed from the soil matrices. The mobility potential of pyrethrin 1 in the examined soils is classified as immobile. (KPIC)

The results from the study conducted by Mori, V. (2015) confirm that the Koc for Pyrethrin 1 is 34674 L/Kg and this value will be used in the environmental risk assessment.

Table A.71 Summary table – Adsorption/desorption

Summary table – Adsorption/desorption									
Method, Guideline, GLP status, Reliability	Soil	Adsorbed AS [%]	K_a	Ka_{OC}	K_d Kd_{OC} K_a/K_d	Kf	1/n	Remarks	**Reference**
USEPA Subdivision N, Section 163-1 GLP Reliability 2	Sandy loam, North Dakota	70.86	268	12472	2332 108679 0.1149	N/A	N/A	N/A	Reynolds & Robinson (1994), Doc III A 7.1.3/01 (KPIC) IIIA-7.2.3.1 (BRA, MGK and SCJ)
	Silty clay loam, Mahaska	75.22	310	16190	1151 60133 0.2693	N/A	N/A	N/A	
	Silt loam, Dundee	77.30	430	74175	2600 448257 0.1654	N/A	N/A	N/A	
	Sand, Wakulla	49.43	198	37847	965 184767 0.2052	N/A	N/A	N/A	
OECD Guideline 121 (Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)) GLP Reliability 1 Key	N/A	N/A	N/A	Pyrethrin 1=34674 Cinerin 1=21136 Jasmolin 1=50119 Pyrethrin 2=7762 Cinerin 2=4732 Jasmolin 2=10471	N/A	N/A	N/A	N/A	Mori, V. (2015), IUCLID 10.1.2 Doc IIIA Section A7.2.3.1

K_a = Adsorption coefficient
Ka_{OC} = Adsorption coefficient based on organic carbon content
K_d = Desorption coefficient
Kd_{OC} = Desorption coefficient based on organic carbon content
K_a / K_d = Adsorption / Desorption distribution coefficient

Table A.72 Summary table – Adsorption/desorption metabolite/ degradant/ transformation- or reaction product*
No data available

Value used in Risk Assessment	
Value/conclusion	Organic carbon/water partition coefficient (Koc)= 34674 L/Kg Log Koc = 4.54
Justification for the value/conclusion	The fate and distribution in the environment was derived from studies on pyrethrin 1, since pyrethrin 1 represents the predominant analogue and a typical member (or paradigm) for the pyrethrum family. Pyrethrin 1 is considered to represent the worst case, as it has a higher Koc value than Pyrethrins 2 and, as the most sensitive compartment is sediment, the adopted approach to exposure assessment is consequently very conservative.

A4.1.2.2 Higher tier soil adsorption studies

No data available.

A4.1.2.3 Volatilisation

Regarding volatilisation, please see Part A, section 1.3 Physical and chemical properties of the active substance.

A.3.1.3. *Bioaccumulation*

Measured aquatic bioconcentration

A bioconcentration test with bluegill sunfish (*Lepomis macrochirus*) exposed to the initial measured concentration of 90.5 ng/L ^{14}C-Pyrethrin 1 under flow-through system was performed. After an uptake phase of 28 days the fish were transferred to clean water for 15 days (depuration phase). After 28 days the bioconcentration factors were determined to be 127, 873 and 471 for edible tissue, non-edible tissue and whole body, respectively. All BCF values refer to the total amount of radioactivity (sum of radiolabelled parent, metabolites and mineralization products). Pyrethrin 1 and two major metabolites (Chrysanthemic acid and another metabolite which was not identified) were separated in the analysis of edible and non-edible (viscera) tissues.

In addition to the BCF steady state the applicant derived a BCF kinetics which resulted in a BCF = 500. This would be the preferred value for risk assessment as indicated in Guidance R7c and also because in the last consecutive three measure points, the test substance concentration in fish varies more than 20%.

The accumulation of Pyrethrin 1 was reversible as approximately half of the [14C] residues from both edible and non-edible tissues had depurated within 1 day of the initiation of the depuration period (half-life was determined to be 1 day). Therefore, the depuration rate was quite fast. (BRA, MGK and SCJ)

Table A.73 Summary table – Measured aquatic bioconcentration

Summary table – Measured aquatic bioconcentration										
Method, Guideline, GLP status, Reliability, Key/supportive study	**Exposure**	**Log Kow of AS**	**Initial concentration of AS**	**Steady state BCF**	**Uptake rate constant (K1)**	**Depur. rate constant (K2)**	**Depur. time (DT_{50})**	**Metabolites**	**Remarks**	**Reference**
OECD 305 US EPA 165-4 GLP Reliability 2 Key	Flow through 28 d	Pyrethrin 1: log Kow = 5.34 (20°C) Pyrethrin 2: log Kow = 3.79 (20°C)	90.5 [14C] Pyrethrin 1 and Pyrethrin 1 (Batch number Radiolabelled: CFQ6932, CFQ7390, CFQ7422; non-radiolabelled: NK9304) (Purity Radiolabelled: 97.0%, 99.6%, 98.1%; non-radiolabelled: 98.9%)	Edible 127X Non-edible 873X Whole Body 479X	Edible 183/1.39 Nonedible 1270/1.36 Whole Body 662/1.32	Edible 1.39 Nonedible 1.36 Whole Body 1.32	1 d	Chrysanthemic acid: %TRR[a] Hexane fraction: 5.4 Methanol fraction: 24.1%TRRb Hexane fraction: 1.2 Methanol fraction: 31.7 Metabolite #3: %TRRb Hexane fraction: 11.6 Methanol fraction: 4.7	-	[redacted] (1994) A7.4.3.3.1/01 (KPIC); IIIA-7.4.2. (BRA, MGK and SCJ) and KPIC

Measured logPow

The partition coefficient n-Octanol-Water log Pow of Pyrethrum Extract components (Pyrethrin 1, Cinerin 1, Jasmolin 1, Pyrethrin 2, Jasmolin 2 and Cinerin 2) was determined by HPLC according to OECD117.

The following logpow values were obtained:

- Pyrethrin 1: 5.59
- Cinerin 1: 5.54
- Jasmolin 1: 6.04
- Pyrethrin 2: 4.32
- Cinerin 2: 4.26
- Jasmolin 2: 4.74

Pyrethrin 1, which was considered the reference component for fate data, has a log kow of 5.59 above the cut-off level for bioaccumulation of the CLP Guidance equal to 4

Conclusion

For bioaccumulation assessment BCF measured values are preferred over logkow values. A BCF value of 500 was calculated, indicating the potential for bioaccumulation of the substance. However, the reported BCF values refer to the total amount of radioactivity (sum of radiolabelled parent, metabolites and mineralization products) and may not reflect the real BCF value of *Chrysanthemum cinerariaefolium* extract from HCS.

Further, a reliable logpow = 5.59 was provided which is above the cut-off value = 4 of the Guidance to determine if a substance is bioaccumulative or not. Based on this value the substance is considered potentially bioaccumulative.

A.3.1.4. *Monitoring data*

No data are available.

A.3.2. Effects on environmental organisms

A.3.2.1. *Aquatic compartment*

A3.2.1.1 Freshwater compartment

Acute/short-term toxicity (freshwater)

FISH

Pyrethrum extract (FEK-99) was tested on Rainbow trout (*Oncorhynchus mykiss*) in a flow-through system during 96 hours at five different nominal concentrations from 1.3 to 10 µg total Pyrethrins/L. Two replicates of ten fish per test concentration were exposed to Pyrethrum extract. This test ([redacted], 1994a) was carried out according to the U.S. EPA Pesticide Assessment Guidelines E, Section 72-1 which is equivalent to the OECD guideline 203 "Fish, Acute Toxicity Test". The test conditions were within the range demanded by OECD 203. Analytical monitoring showed that the measured concentrations were not ≥ 80% of the nominal concentration, thus the mean measured concentrations of total Pyrethrins were used. The 96 hour LC50 was calculated to be 5.2 µg total Pyrethrins/L (with 95% confidence intervals of 3.1 to 5.7 µg total Pyrethrins/L),

based on mean measured concentrations.

Pyrethrum extract (FEK-99) was tested on Bluegill Sunfish (*Lepomis Macrochirus*) system during 96 hours at five different nominal concentrations from 3.2 to 25 µg total Pyrethrins/L (mean measured from 3.1 to 14) . Two replicates of ten fish per test concentration were exposed to Pyrethrum extract. This test ([redacted], 1994b) was carried out according to the U.S. EPA Pesticide Assessment Guidelines E, Section 72-1 which is equivalent to the OECD guideline 203 "Fish, Acute Toxicity Test". From the data collected during the exposition, the 96-hours LC50 was determined to be 10 µg total Pyrethrins/L (95% C.I. of 7.8 to 14 µg/L) based on the mean measured concentrations of total Pyrethrins.

Pyrethrum extract (FEK-99) was tested on Sheepshead minnow (*Cyprinodon variegatus*) in a flow-through system during 96 hours at five nominal concentrations from 10 to 78 µg total Pyrethrins/L. Two replicates of ten fish per test concentration were exposed to Pyrethrum extract. This test ([redacted], 1994c) was carried out according to the U.S. EPA Pesticide Assessment Guidelines E, Section 72-3. From the data collected during this study, the 96-hours LC50 was determined to be 16 µg total Pyrethrins/L (95% C.I. of 14 to 18 µg/L) based on the mean measured concentrations of total Pyrethrins. The abnormal/behavioural effects, mortality, surfacing, loss of equilibrium, fish on the bottom of the test chamber, laboured respiration, dark discoloration, vertical orientation and/or quiescence, were observed in the 18 µg/L test concentration during the study.

INVERTEBRATES

The acute toxicity of Pyrethrins (Pyrethrum extract (FEK-99) to aquatic invertebrates was tested (Putt A.E., 1994a) in *Daphnia magna* with five test concentrations (mean measured concentrations from 2.2 to 14 µg/L). The test was carried out according to the U.S. EPA Pesticide Assessment Guidelines E, Section 72-2 which is equivalent to the OECD guideline 202 "Daphnia sp., Acute Immobilisation Test". The test conditions were within the range demanded by OECD 202. The 48 h EC50 for Pyrethrins was determined to be 12 µg total Pyrethrins/L (10 – 13 µg total Pyrethrins/L). Analytical monitoring of the test substance showed that the measured concentrations were not ≥ 80% of the nominal concentration, thus the mean measured concentrations of total Pyrethrins were used.

In addition the applicants presented data on the toxicity of the substance metabolites (Mantilaci 2015a) and on the toxicity of Pyrethrum Extract (FEK-99) and the 6 Pyrethrin esters (Mantilacci, S. 2015b) to Daphnia magna following OECD Guideline 202 "Daphnia sp., Acute Immobilisation Test and Reproduction Test":

- In the first study, young daphnids (less than 24 hours) are exposed to five pyrethrin metabolites (Pyrethrolone, Cinerolone, Jasmolone, Pyrethric acid, Chrysanthemic acid) at a range of concentrations for a period of 48 hours under static conditions. The test fulfils OECD 202 validity criteria. However, the range of concentrations selected is not enough to reliably estimate an EC50. The highest inhibition, only 40%, at the highest concentration tested, 371.86 µg/L, occurred for Chrysanthemic acid Thus, in most cases, no EC50 was determined in the study and the values were extrapolated outside the range of the tested concentrations. Nevertheless, the test shows that the substance metabolites are less toxic than parent and that monitoring the metabolites during other studies is not necessary. No other effects were observed on the exposed organisms.

- In the second study, the acute immobilisation tests were performed, under semi-static conditions, to assess the effects of Pyrethrum Extract (FEK-99) and the 6 Pyrethrin esters (Pyrethrin 1, Cinerin 1, Jasmolin 1, Pyrethrin 2, Cinerin 2, Jasmolin 2) on Daphnia magna. 20 daphnids less than 24 hours old, were exposed to determinate concentrations of each test item for 48 hours. Probit analysis was used to evaluate the dose-response function and the endpoints with 95% confidence limits.. The test fulfilled validity criteria.

However, test concentrations do not allow to reliably estimating an EC50 for Cinerin 1 and 2, Jasmolin 1 and 2 and Pyrethrin 2. In the test, applied concentrations for Cinerin 1, Pyrethrin 2 and Cinerin 2 only reach a maximum of 45%, whereas for Jasmolin 1 and Jasmolin 2y, 55% inhibitions is reached. For FEK-99 the EC50 = 28.09 µg/L and for Pyrethrin 1 EC50 = 272.81 µg/L.

Despite their deficiencies the above two test showed that metabolites and pyrethrins esters are less toxic than parent.

ALGAE

Unicellular fresh water green alga, *Desmodesmus subspicatus* was exposed under static conditions for 72 hours to six concentrations of Pyrethrum Extract Pale 50 %/L with three replicates of each concentration (Dengler D., 2000). Six replicates of a blank control were run in parallel. Test substance concentrations tested were presented in mg Pyrethrum Extract Pale 50 %/L (from 5.3 to 100). The mean value of the cell concentration was plotted versus time to produce growth curves for Pyrethrum Extract Pale 50 %/L and for the control. This resulted in a NOErC = 30.9 mg/L, an ErC50 = 65.1 mg/L and a EbC50 = 29.0 mg/L (based on nominal concentrations).

However, in the test, due to the physical-chemical properties of Pyrethrum Extract: low water solubility, tendency to bind on surfaces and volatilisation with steam; and because the application procedure was performed by application of the product in acetone to the vessels, volatilisation of the acetone, adding of test medium to the vessels and shaking on flat bed shaker, most of Pyrethrum was bound to the vessel surfaces. The total pyrethrum in the test system was above 74 to 91% of nominal at the beginning and decrease to 61 to 73% at the end of the test. Since the test substance disappears in the test RMS recalculated endpoints based on measured concentrations considering 61% of nominal as a worst case (concentrations were only measured for three concentrations groups and this value maximises concentration loss). This resulted in an EC50 = 39.8 mg/L and EC10 = 19.7mg/L. Transforming this value to total pyretrins an EC50 = 19.mg/L and and EC10 = 9.85mg/L was obtained.

These values are higher than water solubility and the enpoints were considered as water solubility 0.23mg/L.

Table A.77 Summary table – acute/short-term aquatic toxicity

Summary table – acute/short-term aquatic toxicity										
Method, Guideline, GLP status, Reliability, Key/supportive study	**Species**	**Endpoint / Type of test**	**Test material**	**Exposure**		**Results**			**Remarks**	**Reference**
				Design	**Duration**	**NOEC**	**LC/ EC_{10}**	**LC/ EC_{50}**		
Fish										
EPA, Subdivision E, Series 72, § 72-1 GLP Reliability 2 Key	Rainbow trout (*Oncorhynchus mykiss*)	Mortality/ acute	Pyrethrum extract (FEK-99) (Batch R92-254; Purity 57.5%)	Flow-through	96 hours	3.1 µg total pyrethrins/L		5.2 µg total pyrethrins /L	5 concentrations tested, deaths in highest dose group	[redacted] (1994a) A7.4.1.1/01 (BRA, MGK, SCJ) (KPIC)
EPA, Subdivision E, Series 72, § 72-1 GLP Reliability 2	Bluegill sunfish (*Lepomis macrochirus*)	Mortality/ acute	Pyrethrum extract (FEK-99) (Batch R92-254; Purity 57.5%)	Flow-through	96 hours	5.4 µg total pyrethrins /L		10 µg total pyrethrins /L	5 concentrations tested, deaths in the two highest dose groups	[redacted] (1994b) A7.4.1.1/02 (KPIC)
EPA, Subdivision E, Series 72, § 72-3 GLP Reliability 2	Sheepshead minnow (*Cyprinodon variegatus*)	Mortality/ acute	Pyrethrum extract (FEK-99) (Batch R92-254; Purity 57.5%)	Flow-through	96 hours	7.4 µg total pyrethrins /L		16 µg total pyrethrins /L	5 concen-trations tested, deaths in the four highest dose	[redacted] (1994c) A7.4.1.1/ 03 (KPIC

									groups	
Invertebrates										
U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-2 GLP Reliability 2	*Daphnia magna*	Immobility/ acute toxicity	Pyrethrum extract (FEK-99) (Lot. R92-254; 57.48% w/w total Pyrethrins)	Flow through	48h	3.7 µg total pyrethrins /L		12 µg total pyrethrins /L	5 concentrations tested, immobility in the two highest dose groups Measured conc. < 80% of nominal	Putt, A.E. (1994a) IIIA-7.4.1.2 (BRA, MGK, SCJ, KPIC)
OECD Guideline No. 202 GLP Reliability 2	*Daphnia magna*	Immobility/ acute toxicity	Pyrethrolone (98.0 mg/L); Cinerolone (99.9 mg/L); Jasmolone (99.5 mg/L); Pyrethric acid (100.0 mg/L) & Chrysanthemic acid (100.0 mg/L)	Static	48h	Pyrethrolone: ≥393.97 µg/L Cinerolone: ≥367.42 µg/L Jasmolone: ≥398.41 µg/L Pyrethric acid: ≥469.15 µg/L Chrysanthemic acid: 34.92 µg/L	Pyrethrolone: LOEC >393.97 µg/L EC_{10} = 189.78 µg/L Cinerolone: LOEC>367.42 µg/L EC_{10} = 277.80 µg/L Jasmolone: n.d Pyrethric acid:	Pyrethrolone: n.d Cinerolone: n.d Jasmolone: n.d Pyrethric acid: n.d Chrysanthemic acid: 609.95 µg/L	The test fulfils validity criteria. However test concentrations do not allow a reliable estimation of the EC50.	Mantilacci, S. (2015a) Doc IIIA / Section A7.4.1.2 (BRA, MGK, SCJ, KPIC)

							LOEC>469.15 µg/L EC_{10} = 87.34 µg/L Chrysanthemic acid: LOEC = 76.83 µg/L EC_{10} = 85.84 µg/L			
OECD Guideline No. 202 GLP Reliability 2	*Daphnia magna*	Immobility/ acute toxicity	Pyrethrum Extract (FEK-99) (57.03% w/w); Pyrethrin 1 (29.76%); Cinerin 1 (5.55%); Jasmolin 1 (1.81%); Pyrethrin 2 (15.63%); Cinerin 2 (3.18%); Jasmolin 2 (1.10%)	semi-static	48h	Pyrethrum Extract (FEK-99): NOEC: 1.94 µg/L; total Pyrethrins NOEC: 0.89 µg/L Pyrethrin 1: NOEC: 14.09 µg/L Cinerin 1: NOEC: 43.39 µg/L Jasmolin 1: NOEC: 23.48 µg/L Pyrethrin 2: NOEC: 10.67 µg/L Cinerin 2: NOEC: 23.48 µg/L Jasmolin 2: NOEC: 51.65 µg/L	Pyrethrum Extract (FEK-99): LOEC: 4.27 µg/L; total Pyrethrins LOEC: 1.96 µg/L Pyrethrin 1: LOEC: 30.99 µg/L Cinerin 1: LOEC: 95.45 µg/L Jasmolin 1: LOEC: 51.65 µg/L Pyrethrin 2: LOEC: 23.48 µg/L Cinerin 2: LOEC: 51.65 µg/L Jasmolin 2: LOEC: 113.64 µg/L	Pyrethrum Extract (FEK-99) EC_{50} 28.09 µg/L; total Pyrethrins EC_{50} 12.92 µg/L Pyrethrin 1:EC_{50} 61.08 µg/L Cinerin 1: EC_{50} 272.81 µg/L Jasmolin 1: EC_{50} 226.67 µg/L Pyrethrin 2: EC_{50} 262.79 µg/L Cinerin 2: EC_{50} 358.95 µg/L Jasmolin 2: EC_{50} 216.16 µg/L	The test fulfils validity criteria. However, test concentrations only allow to reliably estimate the EC50 for FEK-99 and Pyrethrin 1.	Mantilacci, S. (2015b) Doc IIIA / Section A7.4.1.2
Algae (growth inhibition)[1]						**NOErC**	**EbC50**	**E_rC_{50}**		

OECD 201 EEC Directive C.3 (92/69/EC) GLP Reliability 1	*Desmodesmus subspicatus*	Growth and biomass inhibition	Pyrethrum extract Pyrethrum Extract Pale 50% (Batch 99/11-5 B; Purity 50.17%)	Static	72 hours	0.23 mg/L	0.23 mg/L	0.23 mg/L	6 concen-trations tested, significant inhibitory effects from 30.9 - 100 mg Pyrethrum Pale xtract/L for biomass and growth rate	Dengler D. (2000) A7.4.1.3/01 (KPIC)
Other aquatic plants										
-	-	-	-	-	-	-	-	-	-	-

[1] calculated from growth rate, if not available please include the biomass value (NOEbC/EbCx) or the unspecified NOEC/ECx value

Value used in Risk Assessment	
Value/conclusion	*Oncorhynchus mykiss* EC50 = 5.20 µg total pyrethrins /L = 7.97 µg a.s./L considering the whole extract as the a.s.
Justification for the value/conclusion	Based on acute values, fish (*Oncorhynchus mykiss*) is the most sensitive species tested. The resulting lowest EC50 is 5.20 µg total Pyrethrins/L.

There are some additional tests which were evaluated under PPP regulation for aquatic organisms. They are included in Appendix VII.

Chronic/long-term toxicity (freshwater)

FISH

A flow-through sub-acute toxicity test ([redacted], 1994d) was performed with Fathead minnow (*Pimephales promelas)* during an early life stage exposure. The test was carried out according to the U.S. EPA Pesticide Assessment Guidelines E, Section 72-4 which is equivalent to the OECD guideline 210 "Fish, Early-life Stage Toxicity Test". The test conditions were within the range demanded by OECD 210. Analytical monitoring showed that the measured concentrations were not ≥ 80% of the nominal concentration, thus the mean measured concentrations of total Pyrethrins were used. The NOEC and LOEC were determined to be 1.9 µg total Pyrethrins/l and 3.0 µg total Pyrethrins/l, respectively, based on the effects observed for percent embryo hatch and larval growth (total length and wet weight).

INVERTEBRATES

Toxicity test about effects on reproduction and growth rate was performed with *Daphnia magna* under flow-through conditions (Putt A.E. 1994b). The test was carried out according to the U.S. EPA Pesticide Assessment Guidelines E, Section 72-4 which is equivalent to the OECD guideline 211 "Daphnia magna reproduction test". The test conditions were within the range demanded by OECD 211. Six nominal concentrations were tested from 0 to 4.0 µg total Pyrethrins/L (nominal concentrations of 0, 0.25, 0.50, 1.0, 2.0 and 4.0 µg total Pyrethrins/L). The mean measured concentrations of total Pyrethrins were 0.20, 0.41, 0.86, 2.0 and 3.6 µg/L. The biological results are based on the mean measured concentrations during the test.

The following effects of Pyretrum Extract on daphnids was assessed:

- Immobility of adult daphnids
- Number of living young daphnids
- Number of dead young daphnids
- Appearance and behaviour of adult daphnids at test termination (Day 21)
- Growth (total body length and dry weight)

Survival:

Survival of adult daphnids was determined on test days 0, 1, 2, 4, 7, 9, 11, 14, 16, 18 and 21. Following 11 days of exposure, the mean percent survival of organisms exposed to all treatment levels was within the range of 80 – 100%. Control survival averaged 95%.

Reproduction:

Measurements of offspring production was made on days 0, 1, 2, 4, 7 and three times per week thereafter through study termination (day 21). At each observation interval, the offspring were removed, counted and discarded.

Mean reproduction of 26, 18, 27, 27 and 8 offspring per female was observed at these respective concentrations. Control reproduction averaged 29 offspring per female.
An adverse effect on reproduction was noted in the study, and was considered the most sensitive indicator of the toxicity of pyrethrum extract to *Daphnia magna*. Daphnids exposed to 0.2, 0.41, 0.86, 2.0, and 3.6 µg total Pyrethrins/L released an averaged of 197, 198, 211, 150 and 24 offspring per female, respectively. Statiscal analysis determined a significant reduction in reproduction at the 2.0 and 3.6 µg total Pyrethrins/L concentrations when compared to the reproduction of pooled control daphnids (207 offspring per female). No significant reduction in reproduction was observed at $\leqslant$ 0.86 µg total Pyrethrins/L when compared to the pooled control.

Inmobilitation:

Throughout the exposure period, no young were observed to be immobilized in any test concentration or control.

Body length and weight:

At test termination, the length and weight of each surviving adult daphnia were measured.
Mean total body length of daphnids exposed to 0.2, 0.41, 0.86, 2.0 µg total Pyrethrins/L ranged from 5.32 to 5.46 mm and was statistically reduced as compared to the mean total length of the pool control organisms (5.4 mm). The mean total body length among daphnids exposed to the highest treatment level tested (3.6 µg total Pyrethrins/L) was 4.85 mm and was statistically different from the pooled control. Similarly, mean organism dry weight for daphnids exposed to the four lowest concentrations ranged from 1.47 to 1.52 mg and was statistically comparable to mean weight of the pooled control organisms (1.62 mg). The mean dry weight of daphnids exposed to the highest concentration tested, 3.6 µg total Pyrethrins/L, was 1.09 mg and was statistically reduced as compared to the dry weight of the pooled control organisms. Therefore, the NOEC for growth was determined to be 2.0 µg total Pyrethrins/L.

Comment:
Since there was no statistically significant difference between the survival, reproduction and growth of the organisms in the control and solvent control, the data of survival, reproduction and growth from both control groups were pooled. The pooled control data was used during the comparisons to establish treatment level effects.

Conslusion:
Based on the data of the test, it can be concluded that reproduction was the most sensitive indicator observed during the study.

The EC_{50} was determined to be 3.6 µg total Pyrethrins/l. The LOEC was determined to be 2.0 µg total Pyrethrins/l. The NOEC was determined to be 0.86 µg total Pyrethrins/L, which is the key value.

Table A.78 Summary table – chronic/long-term aquatic toxicity

Summary table – chronic/long-term aquatic toxicity								
Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LOEC/NOEC/EC_{10}[specify the value]		
Fish								
US EPA 72-4 GLP Reliability 2	Fathead minnow (*Pimephales promelas*)	Embryo hatch, survival and growth of larvae	Pyrethrum extract (FEK-99) (Batch R92-254; Purity 57.5%)	Flow-through	35 days	NOEC 1.9 µg total Pyrethrins/L LOEC 3.0 µg total Pyrethrins/L	5 concentrations tested, deaths in all dose groups	[redacted] (1994d) A7.4.3.2 (KPIC and BRA, MGK and SCJ)
Invertebrates								
US EPA 72-4/ GLP Reliability 2 Key	*Daphnia magna*	Reproduction /chronic	Pyrethrum extract (FEK-99) (Batch R92-254; Purity 57.5%)	Flow-through	21 days	NOEC 0.86 µg total Pyrethrins/L LOEC 2.0 µg total Pyrethrins/L EC_{50} (ECx) 3.6 µg total Pyrethrins/L	5 concentrations tested, effects observed in the 2 highest concentrations	Putt A.E. (1994b) A7.4.3.4 (KPIC and BRA, MGK and SCJ)
Algae[1]								
OECD 201 EEC Directive C.3 (92/69/EC) GLP Reliability 2	*Desmodesmus subspicatus*	Growth and biomass inhibition	Pyrethrum extract Pyrethrum Extract Pale 50% (Batch 99/11-5 B; Purity 50.17%)	Static	72 h	NOEC 0.23 mg/L (solubility limit)	6 concentrations tested, significant inhibitory effects from 30.9 - 100 mg Pyrethrum Pale xtract/L for biomass and growth rate	Dengler D. (2000) A7.4.1.3 (KPIC)
other aquatic plants								
-	-	-	-	-	-	-	-	-
1 calculated from growth rate, if not available please include the biomass value (NOEbC/EbCx) or the unspecified NOEC/ECx value								

Value used in Risk Assessment	
Value/conclusion	Daphnia magna NOEC (21 days) = 0.86 µg total pyrethrins/L = 1.32 µg a.s./L, considering the whole extract as the a.s.
Justification for the value/conclusion	Daphnia is the most sensitive species tested. The chronic toxicity to Daphnia magna was determined in a 21-day reproduction study. The resulting NOEC, based on numbers of offspring per adult, was determined to be 0.86 µg total Pyrethrins/L.

A4.2.3.2 Sediment compartment

Acute/short-term toxicity (freshwater sediment)
Three acute immobilisation tests with Chironomus riparius were performed for the chemical similarity report, with pyrethrum extract 49.35%, pyrocide 50% and pyrethrum extract pale 50% (Dabrunz, A., 2017 a, b and c respectively). They all follow OECD guidance 235 "Chironomus sp., Acute Immobilisation Test". In all the three tests, Chironomus riparius larvae (20 in 4 replicates at each concentration) were exposed in 100 mL glass vessels to eight different nominal test concentrations from 0.241 to 60.0 µg total pyrethrins/L for 48 hours under static test conditions. Statistical evaluation was performed with a probit analysis using linear max. likelihood regression to obtain EC10, 20 and 50. The NOEC was established based on the highest test item concentration at which immobilisation was not higher than the acceptable control immobilisation (15 % immobilisation). The endpoints are summarized in table A.79, based on geomean measured concentration. This table shows the lowest endpoint in Dabrunz 2017a study.

In this specific test, observations on immobilization of the *Chironomus riparius* were made after 24 and 48 hours. The immobilised *Chironomus riparius* were counted and abnormal behaviour was noted at test start and every 24 hours thereafter. Water temperature, pH and dissolved oxygen were recorded throughout the exposure period. *Chironomus riparius* were not fed during the test period. Analytical determinations for total Pyrethrins concentration were made from samples taken from each replicate of each test item group at the start and end of the study. Mortality data as absolute numbers of immobile daphnids and as percent of exposed animals is shown below:

Test-Substance Concentration Total Pyrethrins (nominal) [µg/l]	**Immobile *Chironomus riparius* (mean)**			
	Number		**Percentage**	
	24 h	**48 h**	**24 h**	**48 h**
Control	0	0	0	0
Solvent control	0	0	0	0
0.241	1	1	5	5
0.529	1	1	5	5
1.16	4	4	20	20
2.56	6	7	30	35
5.63	7	10	35	50
12.4	7	19	35	95
27.3	13	20	65	100
60.0	17	20	85	100

An EC50 = 3.11 µg total pyrethrins/L based on measured concentration was obtained.Table A.79 Summary table – acute/short-term toxicity to sediment dwelling organisms

Summary table – acute/short-term toxicity to sediment dwelling organisms										
Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results			Remarks	Reference
				Design	Duration	NOEC	LC/EC_{10}	LC/EC_{50}		
OECD 235; GLP GLP Reliability 2 Key	*Chironomus riparius*	Acute immobilisation	Pyrethrum Extract (49.35%)	Static	48 h	0.497 µg total pyrethrins/L	1.04 µg total pyrethrins/L	3.11 µg total pyrethrins/L	Measured concentrations	Dabrunz, A. (2017a) (BRA) Doc IIIA / Section A7.4.3.5.1
OECD 235; GLP GLP Reliability 2	*Chironomus riparius*	Acute immobilisation	Pyrocide® 50%	Static	48 h	2.18 µg total pyrethrins/L	4.79 µg total pyrethrins/L	5.25 µg total pyrethrins/L	Measured concentrations	Dabrunz, A. (2017b) (MGK) Doc IIIA / Section A7.4.3.5.1
OECD 235; GLP Reliability 2	*Chironomus riparius*	Acute immobilisation	Pyrethrum Extract Pale 50%	Static	48 h	3.72 µg total pyrethrins/L	10.0 µg total pyrethrins/L	9.96 µg total pyrethrins/L	Measured concentrations	Dabrunz, A. (2017c) (KPIC) Doc IIIA / Section A7.4.3.5.1

Value used for classification purposes	
Value/conclusion	*Chironomus riparius* EC50 = 3.11 µg total pyrethrins /L = 4.76 µg a.s./L
Justification for the value/conclusion	Based on acute values, this is the most sensitive species tested for water/sediment system.

Chronic/long-term toxicity (freshwater sediment)

The effects of sediment-incorporated test substance on the survival and growth of *Chironomus riparius* was determined under static test conditions during a 28-day exposure period (Thomas, S.T. and Krueger H.O., 2009) in accordance with the OECD test Guideline 218. Larvae of Chironomus riparius were exposed in a static system to nominal concentrations of 31, 63, 125, 250, 500 and 1000 µg.kg-1 of refined Pyrethrum extract FEK 99 (57.03 % w/w total Pyrethrins) in a water-sediment system (spiked sediment) at 20±2ºC. Initial LC50, NOEC y LOEC values submitted by the applicant were based on nominal concentrations. However, analytical monitoring of the test substance, only measured at the lowest and highest test concentration (31 µg Refined Pyrethrum Extract/kg sed. dw and 1000 µg Refined Pyrethrum Extract/kg sed. dw) showed a significant decrease in the test substance concentration over the exposure period. For example, at highest test concentration after 7 days the 11.6% and after 28 days the 0.51% of nominal concentration was found in the sediment. For this reason RMS recalculated endpoints based on measured concentrations resulting in an EC50 >54.6 µg/kg, a LOEC 56.46 µg/kg and a NOEC 28.22 µg/kg.

The concentrations of Pyrethrins were not maintained over the test period, deviating from nominal concentration more than 20 per cent, so the results should be based on measured concentrations (mean recovery of 9.9%).

However, the test presents deviations that make the study not valid, but to be used as supporting information:

- A significant difference occurred only at the highest concentration in the case of the emergence ratio (49% emergence whereas in control it is 74%). It is very likely that this significant difference can be attributed to biological variability. The emergence ratios for concentrations > 1000 µg/kg might not be significantly different to the control. No LC50 could be determined either, due to lack of mortality at the highest test rate supporting the interpretation that the significant effect at the highest rate for the emergence ratio is an outlier. Furthermore there are no confidence intervals given for the emergence ratio.
- The variability of the mean emergence ratios between the different test concentrations indicates that no clear dose-response relationship can be shown. In some cases the lower test rates have even lower or equal emergence ratios compared with the higher test rates. Dunnett's test which may not be the appropriate test due to the high variability of the data (there was a statistically significant difference ($p<0.05$) from the negative control using Dunnett's test).

- The stock solutions appeared slightly cloudy and white with a foamy surface which might be related to residues of detergents used to clean the vessels. The cloudiness increased with increasing concentration. The appearance of the stock solutions might have had an impact on the emergence ratio of the test organisms (being rather a suspension, an homogeneous distribution of the test substance in the sediment is not guaranteed).

In another test, *Chironomus riparius* midge larvae were exposed under static conditions for 28 days to eight concentrations of Pyrethrum Pale Extract (Heintze, 2001) added to the water phase of the system, following OECD test guideline 219. The results were EC 50 for emergence inhibition of the midge, the emergence rate dependent and the development rate dependent NOECs. Nevertheless, this test is not considered for the risk assessment, just as supporting information, as it presents important deficiencies: at the end of the test – after 28 days – the % of total pyrethrins detected in the overlaying

water was ~1 % of initial amount applied, showing a transfer of pyrethrins from water to sediment phase and a subsequent loss from test system by either degradation or volatilization.

In the only reliable test, *Chironomus riparius* midge larvae were exposed under static conditions for 28 days to eight concentrations of Pyrethrum Pale Extract (Gonsior, 2009). Four replicates holding 25 midge larvae each were run. During the period of expected emergence (normally starting at day 10 and lasting until day 25) a daily check of emerged midges was performed. The sex and number of emerging adults were recorded daily. Only the number of fully emerged male and female midges was counted. Test substance concentrations were 1.2, 2.39, 4.78, 9.56, 19.1 and 38.2 mg Pyrethrum Pale Extract 50%/kg sediment dw, and were added to the sediment phase of the system, according to OECD guideline 218.Analytical verification of pyrethrins concentrations in the main test was performed at 1.2 mg Pyrethrum Extract Pale/kg sed. dw, 38.2 mg Pyrethrum Extract Pale/kg sed. dw and solvent control at distinct sampling dates (0, 7 and 28 days after introduction of larvae).

According to the applicant, the EC50 for emergence inhibition of the midge *C. riparius* was estimated to be 6.47 mg Pyrethrum Extract Pale/kg sediment dw (3.24 mg total pyrethrins/kg sed. dw, nominal concentration). The emergence rate dependent NOEC was 4.78 mg Pyrethrum Extract Pale/kg sed. dw equivalent to 2.39 mg totals pyrethrins/kg sed. dw (nominal concentration). The development rate dependent NOEC was also 4.78 mg Pyrethrum Extract Pale/kg equivalent to 2.39 mg total pyrethrins/kg sed. dw (nominal concentration).

eCA recalculated endpoints based on measured concentrations. To consider the decline in test substance concentration, the geometric mean of the measured concentrations for the time 0, day 7 and day 28 for the nominal concentration 0.166 mg Refined Pyrethrum Extract/per vessel calculated. This results in a mean measured concentration of 0.116 mg Refined Pyrethrum Extract/kg sed. Dw vessel of pyrethrum, corresponding to a recovery of 69.88 %. Applying this % recovery to the nominal EC50, NOEC and LOEC, the values of measured endpoints were estimated:

Effect data	28 days [mg Pyrethrum Extract Pale 50 % mg/kg dw nominal]	28 days [mg total pyrethrins mg/kg sediment dw nominal]	measured concentration total pyrethrins mg/kg sediment dw
EC50	6.47	3.24	2.26
NOEC	4.78	2,39	1.67*
LOEC	9.56	4.78	1.29

* The geomean measured concentration results to be 0.116 mg/vessel of pyrethrum, which corresponds to a recovery of 69.88%. Applying this recovery to the nominal NOEC of 4.780 mg/kg dwt of pyrethrum, results in a concentration of 1.67 mg/kg dwt of total pyrethrins.

Table A.80 Summary table – chronic/long-term toxicity to sediment dwelling organisms*

Summary table – chronic toxicity to sediment dwelling organisms								
Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LOEC/NOEC/ EC_{10}[specify the value]		
OECD 218 GLP Reliability 2 Key	*Chironomus riparius*	Developmental time/emergence ratio of midges	Pyrethrum Extract Pale 50% (Batch 2008/7-2; Purity 50.08%)	Static	28 days	NOEC = 4.78 mg/kg dw NOEC=1670 µg a.i./kg dwt total pyrethrins Normalised to 10% Organic carbon content: NOEC= 8350 µg a.i./kg dwt total pyrethrins (from 2%OC) Corrected to wet weight sediment: NOEC= 1815 µg a.i./kg wwt total pyrethrins LOEC = 9.56 [mg/kg dw]; EC_{50} = 6.47 [mg/kg dw]	6 concentrations tested; toxic effects observed in the three highest dose concentrations	Gonsior (2009) A7.4.3.5.1/02 (KPIC)

OECD 218 GLP Reliability 3 Supporting	*Chironomus riparius*	Mortality, growth and behavioural signs	Refined Pyrethrum extract (FEK 99; 57.03% w/w total Pyrethrins)	Static system	28 days	EC_{50} >56.46 µg/kg LOEC 56.46 µg/kg NOEC 28.22 µg/kg	(nominal conc. recalc.to mean measured conc.)	Thomas, S.T. & Krueger, H.O. (2009) IIIA-7.4.3.5.1 (BRA, MGK and SCJ)
OECD 219/ GLP Reliability 3 Supporting	*Chironomus riparius*	Developmental time/emergence ratio of midges	Pyrethrum Extract Pale 50% (Batch 99/11-5 B; Purity 50.17%)	Static	28 days	NOEC=0.0096 mg/L/ LOEC=0.0186 mg/L/ EC_{50}=0.0515 [mg/L]	8 concentrations tested, toxic effects observed in the six highest dose concentrations	Heintze (2001) A7.4.3.5.1 (KPIC)

A4.2.3.3 Marine compartment

Acute/short-term toxicity (seawater)

No data available.

Chronic/long-term toxicity (seawater)

No data available.

A4.2.3.4 Sea sediment compartment

Acute/short-term toxicity (sea sediment)

No data available.

Chronic/long-term toxicity (sea sediment)

No data available.

A4.2.3.5 Higher tier studies on aquatic organisms

No data available.

A.3.3. Overall summary of acute and chronic aquatic toxicity data and Comparison with the CLP criteria

Chrysanthemum cinerariaefolium extract from HCS is a complex substance of natural origin. Its main active components are Pyrethrin I: Pyrethrin 1 (min 418.9 g/kg), Cinerin 1 (min 46.0 g/kg), Jasmolin 1. (min 28.8 g/kg); Pyrethrin II: Pyrethrin 2 (min 285.5 g/kg), Cinerin 2 (min 41g/kg) and Jasmolin 2 (min 21.8g/kg). As a main component of the mixture, Pyrethrin 1 has been considered as a surrogate for many of the fate data. Further the substance contains other plant material (max 88.1 g/kg), BHT (max 69.4 g/kg), water (max 2.7 g/kg) and a solvent when commercialised. These other components are not relevant from an ecotoxicological point of view. The substance is stable without the solvent. Hence, here, a classification is provided with and without the solvent and worst case outcome is proposed for classification.

A.3.3.1. *Short-term (acute) aquatic hazard*

The hazard categories for acute aquatic toxicity and their related criteria are set out in Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Annex I, Section 4.1.

In this case, *Chrysanthemum cinerariaefolium* extract from HCS should be considered as a UVCB (plant extract), and hence, be classified based on test data of a mixture as a whole. Please see Appendix VI for further information on the active substance as a UVCB.

According to Annex I: 4.1.3.3.1 of EC 1272/2008, "when the mixture as a whole has been tested to determine its aquatic toxicity, this information can be used for classifying the mixture."

There are adequate toxicity data for the a.s. (UVCB) for fish, invertebrates and algae. In addition, there is data for Chironomus based on OECD 235 which is a test done in water-only vessels and hence valid for classification. The use of Chironomus riparius values is further justified by the insecticidal mode of action of the substance. According to Table 4.1.0.(a):

- Being *Chironomus riparius* the most vulnerable species with a LC50 = 0.00311 mg total pyrethrins/l which is equivalent to 0.00476 mg/l of *Chrysanthemum cinerariaefolium* extract from HCS, without solvent (pyrethrins are at a

concentration of 65.27% in the composition of the plant extract considered as the mixture in the representative source), in table 4.1.0 (a), if LC50 < 1 mg/l, then category acute 1 applies.

- In Annex I, table 4.1.3, if 0.001<LC50<0.01, then a multiplying factor M = 100 applies.

Chrysanthemum cinerariaefolium extract from HCS is classified as Aquatic Acute 1; H400, M=100.

A.3.3.2. *Chronic/ long-term aquatic hazard (including information on bioaccumulation and degradation)*

Long-term toxicity:

- There are adequate chronic toxicity data for the three trophic levels for the mixture as a whole (plant extract). In addition, there is also chronic data for *Chironomus riparius,* the most sensitive acute species.
- It can be concluded that the most sensitive aquatic organisms, on the basis of their chronic toxicity endpoints, is *Daphnia magna* (lowest NOEC = 0.00086 mg/l).

Bioaccumulation:

According to the CLP Guideline the critera for determining if a substance is potentially bioaccumulative is the following:

Valid/high quality experimentally determined BCF value → YES:
→ BCF ≥ 500: *The substance meets the criterion*
→ BCF < 500: *The substance does not meet the criterion*
Valid/high quality experimentally determined BCF value → NO:
→ Valid/high quality experimentally determined log Kow value → YES:
→ log Kow ≥ 4: *The substance meets the criterion*
→l og Kow < 4: *The substance does not meet the criterion*

Reliable BCF measured values are preferred over logkow values. A BCF value of 500 was calculated, indicating the potential for bioaccumulation of the substance. However, the reported BCF values refer to the total amount of radioactivity (sum of radiolabelled parent, metabolites and mineralization products) and may not reflect the real BCF value of *Chrysanthemum cinerariaefolium* extract from HCS.

Further a reliable logpow = 5.59 was provided which is above the cut-off value = 4. Based on this value the substance is considered potentially bioaccumulative.

Degradation:

According to the guidance on the application of CLP criteria (2017), the substance is considered to be non-rapidly degradable unless at least one of the following is fulfilled:

a. The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability. The pass level of the test (70 % DOC removal or 60 % theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation; or .
b. The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of <16 days (corresponding to a degradation of >70 % within 28 days); or
c. The substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydrolysis, in the aquatic environment with a half-life <16 days (corresponding to a degradation of >70% within 28 days), and it can be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment

Chrysanthemum cinerariaefolium extract from HCS is a complex substance of natural origin. According to the Guidance on the Application of the CLP criteria (version 5, July 2017) a complex substance, such as UVCBs, should be regarded as not rapidly degradable if the constituents that are not rapidly degradable constitute a significant part of the substance, e.g. more than 20%, or for a hazardous constituent an even lower content.

Chrysanthemum cinerariaefolium extract from HCS contains 43.9% of Pyrethrin 1. Hydrolysis data for this component yields half-lives > 16 days across different pHs at 25ºC. According to the Guidance on the Application of the CLP criteria (version 5, July 2017), data on hydrolysis might be considered for classification purposes only when the longest half-life determined within the pH range 4-9 is shorter than 16 days. Thus, hydrolysis cannot be considered for classification purposes in this case.

In water simulation tests Pyrethrin 1 was not ultimately degraded, not meeting the guideline requirement of with a half-life <<16 days (corresponding to a degradation of > 70 % within 28 days), being the half-life around 18 days at 12ºC.

In water/sediment the substance primary degraded with DT50 values ranging from 1.6 to of 10.5 days and transformed into metabolites harzardous to the aquatic environment or non-identified metabolites. Hence, it cannot be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment

Further, results of the standard test indicate that Pyrethrum Extract is not readily biodegradable as CO_2 production accounted for 46% by the end of the test on day 29 (section A4.1.1.2.1 Biodegradability (ready/inherent)).

Based on the above *Chrysanthemum cinerariaefolium* extract from HCS is considered not rapidly degradable (NRD).

The hazard categories for chronic aquatic toxicity and their related criteria are set out in Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Annex I, Section 4.1, Table 4.1.0.(b). According to table 4.1.0 (b)(i), for non-rapidly degradable substances, for which there are adequate chronic toxicity data available:

- For *Daphnia magna* there is the lowest NOEC = 0.00086 mg total pyrethrins/l, equivalent to 0.00132 mg *Chrysanthemum cinerariaefolium* extract from HCS, without solvent. If NOEC< 0.1 mg/l, then category chronic 1 applies.
- In Annex I, table 4.1.3, if 0.001<NOEC<0.01, a multiplying factor M = 10 applies for NRD substances.

Chrysanthemum cinerariaefolium extract from HCS is classified as Aquatic Chronic 1; H410; M=10 (when considering the substance as total pyrethrins the M factor would be 100 based on a NOEC = 0.00086 mg/L).

In conclusion, Acute 1, M=100, Chronic 1, M= 10 is the proposed classification for *Chrysanthemum cinerariaefolium*, extract from open and mature flowers of *Tanacetum cinerariaefolium* obtained with hydrocarbon solvents.

A.3.3.3. *Conclusion on classification and labelling for environmental hazards and comparison with the CLP criteria*

Chrysanthemum cinerariaefolium extract from HCS should be classified with respect to the environment as Aquatic Acute 1, H400, M = 100 and Aquatic Chronic 1, H410, M = 10.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

Chrysanthemum cinerariaefolium extract obtained with hydrocarbon solvent is a complex substance of natural origin. The DS indicates that according to the Guidance on the Application of the CLP criteria, complex substances such as UVCBs should be regarded as not rapidly degradable if the constituents that are not rapidly degradable constitute a significant part of the substance, e.g. more than 20 %, or for a hazardous constituent an even lower content.

Chrysanthemum cinerariaefolium extract obtained with hydrocarbon solvent fate is represented by pyrethrin 1. Hydrolysis data for this component yields half-lives > 16 days across different pHs at 25ºC. Following to the Guidance on the Application of the CLP criteria, the DS considered that data on hydrolysis might be considered for classification purposes only when the longest half-life determined within the pH range 4-9 is shorter than 16 days. Thus, hydrolysis cannot be considered for classification purposes in this case.

In water tests pyrethrin 1 was not ultimately degraded, and the guideline requirement of a half-life < 16 days was not met (corresponding to a degradation of > 70 % within 28 days).

In water/sediment the substance primary degraded with DT_{50} values ranging from 1.62 to 10.5 days at tests temperature and transformed into metabolites hazardous to the aquatic environment or non-identified metabolites.

Further, ready biodegradation available for Pyrethrum Extract showed that the substance is not readily biodegradable.

Based on the above *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent was considered **not rapidly degradable**.

Bioaccumulation

For bioaccumulation assessment, the DS reported that BCF value of 500 was calculated from a test on *Lepomis macrochirus* conducted according to the OECD TG 305, indicating the potential for bioaccumulation of the substance. However, the reported BCF values refer to the total amount of radioactivity (sum of radiolabelled parent, metabolites and mineralization products) and the DS concluded that these values may not reflect the real BCF value of *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent.

Further, a reliable Log K_{ow} = 5.59 was reported by the DS which is above the cut-off value of 4 set in the CLP Regulation to determine if a substance is bioaccumulative or not. Based on this value, the DS concluded that the substance is considered potentially bioaccumulative.

Aquatic toxicity

Acute Aquatic Toxicity

The DS presented toxicity data for fish, invertebrates and algae. The resulting lowest EC_{50} was for *Chironomus* based on OECD TG 235, which was a test done in water-only vessels and can therefore be used directly for classification. The DS considered that the use of *Chironomus riparius* data further justified by the insecticidal mode of action of the substance.

Chironomus riparius was the most sensitive species with an LC_{50} = 0.00311 mg total pyrethrins/L obtained from a test performed according to OECD TG 235, which is equivalent to 0.00476 mg/L of *Chrysanthemum cinerariaefolium* extract from hydrocarbon solvent extraction, without solvent (pyrethrins are at a concentration of 65.27 % in the composition of the plant extract considered as the mixture in the representative source). In accordance with table 4.1.0 (a) of CLP Regulation, if LC_{50} < 1 mg/L, Aquatic Acute 1 is warranted.

Considering the Annex I to the CLP Regulation, table 4.1.3, the DS considered that as 0.001 < LC_{50} ≤ 0.01, then a multiplying factor M = 100 applies and *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent is classified as **Aquatic Acute 1; H400, M = 100**.

Chronic Aquatic Toxicity

For chronic toxicity, the DS reported data for the three trophic levels for the mixture as a whole (plant extract). The most sensitive aquatic organism was *Daphnia magna* (lowest NOEC = 0.00086 mg total pyrethrins/L). In addition, there is also chronic data for *Chironomus riparius*, the most sensitive species under acute testing.

According to the Guidance on the Application of the CLP criteria a complex substance, such as UVCBs, should be regarded as not rapidly degradable if the constituents that are not rapidly degradable constitute a significant part of the substance, e.g., more than 20 %, or for a hazardous constituent an even lower content. Therefore, the DS concluded that as *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent is considered as not rapidly degradable (NRD) and based on *Daphnia magna* NOEC = 0.00086 mg total pyrethrins/L, equivalent to 0.00132 mg *Chrysanthemum cinerariaefolium* extract from hydrocarbon solvent extraction, without solvent, classification as **Aquatic Chronic 1; H410; M = 10** (when considering the substance as total pyrethrins the M-factor would be 100 based on a NOEC = 0.00086 mg/L) was warranted according to tables 4.1.0(b)(i) and 4.1.3 in Annex I to the CLP Regulation.

Overall, a classification as Aquatic Acute 1, M = 100, and Aquatic Chronic 1, M = 10 was proposed by the DS for *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent.

Comments received during consultation

During the consultation one MS agreed the classification proposed by the DS. Another asked for modifications of the CLH report regarding several mistakes and noticed that

an acute toxicity study with *Hyalella azteca* with an LC_{50} of 0.00076 mg total pyrethrins/L, which is equivalent to 0.00092 mg/L of *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent, without solvent, was available and would lead to an M-Factor of 1000. For the chronic aquatic toxicity, a study on *Americamysis bahia* with a NOEC of 0.00025 mg total pyrethrins/L which is equivalent to 0.00030 mg/L of *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent, was also available, leading to an M-Factor of 100. Regarding these data, the MS considered that the classification proposal should take into account all available and reliable data, the M-factor should be derived based on these lower effect concentrations and then the classification should be adjusted. Based on the studies with *A. bahia*, the DS proposed to consider the higher M-factor for the chronic classification (M-Factor 100 for not readily biodegradable substances, based on *A. bahia* study in the interval 0.0001 < NOEC < 0.001 mg/L) and for acute classification, the M-factor would be 100, same as in the initial proposal. The DS was not in favour of an acute M-factor of 1000, based on a study with *H. azteca*, as no OECD guideline is adopted for this species, and there are some deficiencies stated in the RAR (the analytical methods not acceptable, the composition of batch remained uncharacterized, and the study is not accepted by method experts).

A National Authority commented on the classification proposal and emphasized that due to the lack of long-term toxicity data for the most sensitive fish species *Oncorhynchus mykiss* and for *Chironomus riparius*, the surrogate approach should be considered which would result in a more stringent M-factor of 100. The DS considered that the three trophic level chronic studies submitted were valid for classification purpose and agreed that the M-factor of 100 for chronic classification should be based on a more stringent NOEC obtained for other invertebrates studied.

Assessment and comparison with the classification criteria

Degradation

Abiotic degradation

***Table**: Summary of valid studies regarding abiotic degradation*

Method, Guideline	Initial TS concentration	Results	Remarks	Reference
US-EPA Pesticide Assessment Guidelines, subdivision N, Series 161-1 GLP Reliability 2	14C-pyrethrin 1 (Batch number CFQ.7422; Purity 98.1 %) From 0.31 to 0.38 mg ^{14}C-pyrethrin 1/L	DT_{50} = 687 days at pH 5, 25°C DT_{50} = 527 days at pH 7, 25°C DT_{50} = 17 days at pH 9, 25°C DT_{50} = 1476 days at pH 7, 12°C	Supportive study	Selim S. (1995) IIIA-7.1.1.1.1 (BRA, MGK and SCJ) Doc III A7.1.1.1.1 (KPIC)
OECD TG 111 (Hydrolysis as a Function of pH) GLP	pyrethrin 1 Lot/Batch: XX-82-P1	DT_{50} ranged from 0.4 to 211.6 days DT_{50} for pyrethrin 1 at	**Key study**	Perboni, A. (2015) IUCLID 10.1.1.1.a (BRA, MGK, SCJ and KPIC)

Reliability 1	Purity:99.4 % pyrethrin 2 Lot/Batch: XX-82-P2 Purity: 99.3 % cinerin 1 Lot/Batch: XX-82-C1 Purity: 97.5 % cinerin 2 Lot/Batch: XX-82-C2 Purity: 99.3 % jasmolin 1 Lot/Batch: XX-82-J1 Purity: 99.3 % jasmolin 2 Lot/Batch: XX-82-J2 Purity: 96.6 % 0.02 mg/L for each analyte	25°C is 115 d at pH 7. At 25ºC and pH 9 values for DT_{50} ranged from 4.2 to 14.9 days.		
US-EPA Pesticide Assessment Guidelines, Subdivision N, Series 161-2 GLP Reliability 1	Radiolabelled pyrethrin 1, product code CFQ.7422, Purity 98.1 % Non-radiolabelled pyrethrin 1, product code NK9212, Purity 97 % Buffer solution (pH 7): mean overall recovery = 97.4 % (range: 91.3 to 102.5 %)	DT_{50} = 11.8 h (^{14}C-pyrethrin 1 + E-isomer)	**Key study**	Selim, S. (1995) and Werle, H. (1991) Doc III IIIA-7.1.1.1.2 (KPIC, BRA, MGK and SCJ)

Hydrolysis

Two valid studies were presented and indicated that pyrethrin 1 is hydrolytically stable at pH5 and 7. At pH 9, a degradant (known only as 'A') increased in proportion to the decrease of pyrethrin 1, accounting for 61 % of the applied radioactivity after 30 days. Another study performed according to OECD 111 assessed abiotic hydrolytic transformations of the 6 Pyrethrum extract components (pyrethrin 1, cinerin 1, jasmolin 1, pyrethrin 2, cinerin 2 and jasmolin 2) in an aquatic system at pH 4-9 under sterile conditions in the absence of light. At pH 4 significant but slow hydrolysis was only observed for some components. At pH 7 relevant hydrolysis was determined for all components, but generally only at elevated temperatures. At pH 9 significant hydrolysis was shown for all six components at all temperatures, resulting in fastest hydrolysis at 45°C.

Phototransformation in water

A photolysis test with Trans-[cyclopropane-1-^{14}C] pyrethrin 1 was performed according to the US EPA Pesticide Assessment Guidelines, Subdivision N, 161-2 (comparable to

the OECD TG 316 "Phototransformation of chemicals in water - direct photolysis"). Results showed that the photolysis rate of pyrethrin 1 is consistent with first order kinetic with a half-life of 11.8 hours of sunlight.

Biodegradation

***Table**: Summary of valid biodegradation studies presented in the CLH report*

Method, Guideline, GLP status, Reliability, Key/supportive study	**Test type**	**Test substance concentration**	**Degradation**	**Remarks**	**Reference**
OECD TG 301 B GLP Ready Biodegradability Reliability 2	CO_2 evolution test	[Refined Pyrethrum Extract (Batch number FEK-99; purity 57.03 %) 10 mL sludge/L mineral medium	**46 % after 29 days** **Not readily biodegradable**	Key study	Barnes, S. (200 IIIA- 7.1.1.2.1 (MGK, BRA ar SCJ)1.2.1.1.1
OECD TG 301 B GLP Reliability 2	CO_2 evolution test	Pyrethrum Extract (Batch 94/10.7; Purity 25.14 % total pyrethrins) 10 mL sludge/L mineral medium	**4.6 % after 28 days** **Not readily biodegradable**	Key study	Koopmans (1995), Doc I A7.1.1.2.1 (KPIC)
OECD TG 309 GLP Reliability 1		[cyclopentenone-2-^{14}C] pyrethrin 1, (Batch CFQ42752; Purity >97.7 %) 10 µg/L (i.e. "low dose") and 100 µg/L (i.e. "high dose")	darkness under aerobic conditions in the laboratory at 20 ± 2°C **Mineralisation 7 % after 62 days**	The major degradation product was pyrethrolone which reached a maximum of 9.5 % AR after 21 days and then decreased to 2.8 % AR at the last sampling interval.	Hein W. *et al*., 2017, IUCLID 10.1.3.2 Doc IIIA / Section 7.1.2.1.1.1
US EPA Subdiv. N, § 162-4 Chemistry: Environmental Fate GLP Reliability 2	Water/sediment pond	^{14}C-pyrethrin 1 (Batch number CFQ 7390; Purity 98.7 % - 99.7 %) ca. 10 ppm	**Mineralisation 4 % after 30 days** **DT50 (25°C) = 10.5 days** **DT50 (12°C) = 29.7 days** Major metabolite: chrysanthemic acid : maximum occurrence in the water/sediment system of 21.2 % of applied radioactivity on day 21 three additional	Key study only one sediment was used for determination of degradation rates whereas Guidance indicates that at least two sediments the test finished before guidance indicates, at day 30, when still 14.7 % of the substance was present.	Robinson, R. A Wisocky, M.J. (1994), Doc III A7.1.2.2.2 (KPIC) (MGK, BRA and SCJ)

			minor degradates. were detected at various intervals, none exceeding 5 % of the initial concentration of pyrethrin 1 at any point		
OECD TG 308 (April 2002), SETAC 1995 GLP Reliability 2 Key	Water/sediment pond (Ensingen, district of Enz, Germany Sandy silt) Creek (Spiegelberg, district of Rems-Murr, Germany Sand)	56.0 µg ^{14}C-pyrethrin 1 (56.6 µg unlabelled pyrethrin 1) [cyclopropane-1-^{14}C] pyrethrin 1 (Lot No. CFQ14811 Batch 1); Purity Radiochemical 98.4 %) Pyrethrum Pale Extract (Batch number 2006/3-3/Pale; Purity pyrethrins I: 30.65 % w/w pyrethrins II: 19.49 % w/w Total pyrethrins: 50.14 % w/w)	Mineralisation 30-51 % at the end of the test in the whole water/sediment system pyrethrin 1 DT50 ranged from 1.6 days (silt) to 2.4 days (sand) chrysanthemic acid DT50 =18 to 109 days, respectively	Key study	- Witte A. (2007), Doc III A7.1.2.2.2/02 (KPIC)
US EPA Pesticide Assessment Guidelines, Subdivision N, Series 162 – 3 GLP Reliability 2	Water/sediment Sandy loam	Trans-[cyclopropane-1-^{14}C] pyrethrin 1 (Batch NB8309; > 97.99 % (radiochemical purity)) 10 mg/L	DT50 = 86 days for the whole system (240.8 days reflecting the average EU outdoor temperature of 12ºC)	Key study	Robinson, R.A. and Wisocky, M.J. (1995) IIIA-7.1.2.2.2 (BRA, MGK and SCJ)

Two biodegradation studies according to OECD TG 301B (CO_2 evolution test) are available. In Barnes (2002), the Refined Pyrethrum Extract was degraded for 46 % after 29 days. In Koopmans (1995), Pyrethrum Extract was poorly degraded (<10 % degradation of Pyrethrum Extract). Results of these studies indicate that Pyrethrum extract is neither readily nor inherently biodegradable.

Aerobic aquatic degradation

Data on degradation rate and metabolism of pyrethrin 1 in natural water were presented in an OECD TG 309 study. In Hein and Moendel (2017), degradation rates (DT50, DT90), metabolism and identification of transformation products in water including a mass balance was determined. In the test, calculated SFO DT50 values for pyrethrin 1 ranged from 6.7-10.7 days (at 20 ± 2°C). The main degradation product was pyrethrolone which reached a maximum of 9.5 % AR after 21 days and then decreased to 2.8 % AR at the last sampling interval. Several non-identified fractions were detected but these were minor and/or composed of several peaks. Mineralisation reached a maximum of 7 % by the end of the study.

Water/sediment degradation test

Robinson and Wisocky (1994) performed water/sediment simulation test to degrade pyrethrin 1 in an aerobic aquatic environment. Degradation proceeded initially by oxidation to form chrysanthemic acid and a number of low level degradants. Residues in water and sediment were initially extracted but extended degradation was accompanied by the formation of residues that were bound to sediment humus fractions and appeared to be partially comprised of bound chrysanthemic acid. Mineralisation was minor, 4 % CO_2 at day 30. The half-life of the pyrethrin 1 in the water/sediment system tested was calculated to be 10.5 days following pseudo-first-order kinetics at 25 ºC. This is equivalent to 29.7 days at 12 ºC.

In a second study conducted by Witte (2007), pyrethrin 1 was observed to degrade rapidly when applied to two separate water/sediment systems taken from the natural environment. This occurred via a rapid movement from the water phase into the sediment phase combined with a steadily increasing mineralization to CO_2 (30 – 51 % at test end) and breakdown in both aquatic and sediment phases to the metabolite chrysanthemic acid (maximum 65.6 and 66.8 %). The half-lives for pyrethrin 1 and chrysanthemic acid in the whole water/sediment system ranged from 1.6 to 2.4 days and 18 to 109 days, respectively.

The anaerobic metabolism of ^{14}C-Pyrethrin was studied under laboratory conditions in a water sediment model system at an initial concentration of 10 mg/L at 25ºC (Robinson and Wisocky 1995). In this study ^{14}C-Pyrethrin dissipated under anaerobic aquatic sediment conditions with a calculated half-life of 86 days for the whole system (240.8 days reflecting the average EU outdoor temperature of 12ºC).

Conclusion

Chrysanthemum cinerariaefolium extract obtained with hydrocarbon solvent is a complex substance and according to the Guidance on the Application of the CLP criteria, when the constituents that are not-rapidly-degradable constitute a significant part of the complex substance e.g. more than 20 %, or for a hazardous constituent, an even lower content, the substance should be regarded as not rapidly degradable. *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent fate is represented by pyrethrin 1. Regarding that:

- Hydrolysis data for this component yields half-lives > 16 days across different pHs at 25ºC.
- In water tests pyrethrin 1 was not ultimately degraded, not meeting the guideline requirement of a half-life <16 days (corresponding to a degradation of > 70 % within 28 days).
- In water/sediment the substance primary degraded with DT50 values ranging from 1.62 to 10.5 days at tests temperature and transformed into metabolites hazardous to the aquatic environment or non-identified metabolites.
- And that ready biodegradation available for Pyrethrum Extract showed that the substance is not readily biodegradable.

RAC concurs with the DS to consider the *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent as **not rapidly degradable.**

Bioaccumulation

In a valid OECD TG 305 study (Anonymous, 1994), the bioconcentration ^{14}C-pyrethrin 1 in the bluegill sunfish (*Lepomis macrochirus*) under flow-through system was investigated. After 28 days exposure, the bioconcentration factors were determined to be 127, 873 and 471 for edible tissue, non-edible tissue and whole body, respectively. In addition to the BCF steady state, a BCF kinetics was derived and a BCF = 500 was calculated.

The partition coefficient n-Octanol-Water log K_{ow} of Pyrethrum Extract components (pyrethrin 1, cinerin 1, jasmolin 1, pyrethrin 2, jasmolin 2 and cinerin 2) was determined by HPLC according to OECD TG 117.

The following Log K_{ow} values were obtained:

- pyrethrin 1: 5.59
- cinerin 1: 5.54
- jasmolin 1: 6.04
- pyrethrin 2: 4.32
- cinerin 2: 4.26
- jasmolin 2: 4.74

Pyrethrin 1, which was considered the reference component for fate data, has a log K_{ow} of 5.59 above the CLP cut-off of 4.

Conclusion

Regarding the calculated BCF value of 500, and a reliable log K_{ow} = 5.59 calculated for pyrethrin 1 which is above the cut-off value = 4, RAC concurs with the DS to consider the substance as **potentially bioaccumulative**.

Aquatic Toxicity

In the CLH report section 1.1, the UVCB is defined as pyrethrins, which may be divided into the two groups pyrethrins I (consisting of pyrethrin 1, cinerin 1, and jasmolin 1) and pyrethrins II (consisting of pyrethrin 2, cinerin 2 and jasmolin 2). The UVCB contains plant material, 2,6-di-tert-butyl-p-cresol (BHT), and water. For ecotoxicity studies, used test materials are UVCBs as Pyrethrum Stewardship Blend containing total pyrethrins. Compared to this material, other constituents, including solvent, are not relevant. Therefore, available endpoint data is based on measured values of total pyrethrins. In the CLH report section A.3.3, the quantities are defined as total pyrethrins 84.2 %, other components are not relevant for classification. The constituent factor stated in the respective CLH reports section A.3.3.1 (65.27) appears incorrect. Recalculating the endpoints to 100 % is not necessary as total pyrethrins are > 80 %. Then, the aquatic toxicity endpoints are expressed in total pyrethrins and compared with the CLP threshold defined for aquatic environment classification purposes.

Acute aquatic toxicity

***Table**: Summary of valid acute aquatic toxicity tests*

Guideline	Species	Endpoint	Test material	Design	Duration	LC/ EC_{50}	Remarks	Reference
Fish								
EPA, Subdivision E, Series 72, § 72-1 GLP Reliability 2 Key	Rainbow trout (*Oncorhynchus mykiss*)	Mortality/ acute	Pyrethrum extract (FEK-99) (Batch R92-254; Purity 57.5 %)	Flow-through	96 hours	0.0052 mg total pyrethrins/L	5 concentrations tested, deaths in highest dose group	Anonymous (1994a) A7.4.1.1/01 (BRA, MGK, SCJ) (KPIC)
EPA, Subdivision E, Series 72, § 72-1 GLP Reliability 2	Bluegill sunfish (*Lepomis macrochirus*)	Mortality/ acute	Pyrethrum extract (FEK-99) (Batch R92-254; Purity 57.5 %)	Flow-through	96 hours	0.010 mg total pyrethrins/L	5 concentrations tested, deaths in the two highest dose groups	Anonymous (1994b) A7.4.1.1/02 (KPIC)
EPA, Subdivision E, Series 72, § 72-3 GLP Reliability 2	Sheepshead minnow (*Cyprinodon variegatus*)	Mortality/ acute	Pyrethrum extract (FEK-99) (Batch R92-254; Purity 57.5 %)	Flow-through	96 hours	0.016 mg total pyrethrins/L	5 concentrations tested, deaths in the four highest dose groups	Anonymous (1994c) A7.4.1.1/03 (KPIC)
Invertebrates								
U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-2 GLP Reliability 2	*Daphnia magna*	Immobility/ acute toxicity	Pyrethrum extract (FEK-99) (Lot. R92-254; 57.48 % w/w total pyrethrins)	Flow through	48h	0.012 mg total pyrethrins/L	5 concentrations tested, immobility in the two highest dose groups Measured conc. < 80 % of nominal	Putt, A.E. (1994a) IIIA-7.4.1.2 (BRA, MGK, SCJ, KPIC)
OECD TG 202; GLP Reliability 2	*Daphnia magna*	Immobility/ acute toxicity	Pyrethrolone (98.0 mg/L); Cinerolone (99.9 mg/L); Jasmolone (99.5 mg/L); Pyrethric acid (100.0 mg/L) & Chrysanthemic acid (100.0 mg/L)	Static	48h	Pyrethrolone: n.d Cinerolone: n.d Jasmolone: n.d Pyrethric acid: n.d Chrysanthemic acid: 0.610 mg/L	The test fulfils validity criteria. However test concentrations do not allow a reliable estimation of the EC50.	Mantilacci, S. (2015a) Doc IIIA / Section A7.4.1.2 (BRA, MGK, SCJ, KPIC)
OECD TG 202; GLP	*Daphnia magna*	Immobility/ acute toxicity	Pyrethrum Extract (FEK-99) (57.03 %	semi-static	48h	Pyrethrum Extract (FEK-99) EC_{50}	The test fulfils validity	Mantilacci, S. (2015b)

Reliability 2			w/w); pyrethrin 1 (29.76 %); cinerin 1 (5.55 %); jasmolin 1 (1.81 %); pyrethrin 2 (15.63 %); cinerin 2 (3.18 %); jasmolin 2 (1.10 %)			0.028 mg/L; total pyrethrins EC_{50} 0.013 mg/L pyrethrin 1:EC_{50} 0.061 mg/L cinerin 1: EC_{50} 0.272 mg/L jasmolin 1: EC_{50} 0.227 mg/L pyrethrin 2: EC_{50} 0.263 mg/L cinerin 2: EC_{50} 0.359 mg/L jasmolin 2: EC_{50} 0.216 mg/L	criteria. However, test concentrations only allow to reliably estimate the EC50 for FEK-99 and pyrethrin 1.	Doc IIIA / Section A7.4.1.2
Algae (growth inhibition)								
OECD TG 201; GLP Reliability 1	*Desmodesmus subspicatus*	Growth and biomass inhibition	Pyrethrum extract Pyrethrum Extract Pale 50 % (Batch 99/11-5 B; Purity 50.17 %)	Static	72 hours	0.23 mg/L (solubility limit)	6 concen-trations tested, significant inhibitory effects from 30.9 - 100 mg Pyrethrum Pale xtract/L for biomass and growth rate	Dengler D. (2000) A7.4.1.3/01 (KPIC)
Sediment dwelling organisms								
OECD TG 235; GLP Reliability 2 Key	*Chironomus riparius*	Acute immobilisation	Pyrethrum Extract (49.35 %)	Static	48 h	0.00311 mg total pyrethrins/L	Measured concentrations	Dabrunz, A. (2017a) (BRA) Doc IIIA / Section A7.4.3.5.1
OECD TG 235; GLP Reliability 2	*Chironomus riparius*	Acute immobilisation	Pyrocide® 50 %	Static	48 h	0.00525 mg total pyrethrins/L	Measured concentrations	Dabrunz, A. (2017b) (MGK) Doc IIIA / Section A7.4.3.5.1
OECD TG 235; GLP Reliability 2	*Chironomus riparius*	Acute immobilisation	Pyrethrum Extract Pale 50 %	Static	48 h	0.00996 mg total pyrethrins/L	Measured concentrations	Dabrunz, A. (2017c) (KPIC) Doc IIIA / Section A7.4.3.5.1

Valid acute toxicity tests are available for all the three trophic levels. In three studies, the Pyrethrum extract (FEK-99) was tested on the Rainbow trout (*Oncorhynchus mykiss*), Bluegill sunfish (*Lepomis macrochirus*) and the Sheepshead minnow (*Cyprinodon variegatus*) in flow-through systems during 96 hours at five different nominal concentrations of pyrethrins, following to the U.S. EPA Pesticide Assessment Guidelines E, Section 72-1 which is equivalent to the OECD TG 203. The 96 hour LC_{50} was calculated to be 0.0052 mg total pyrethrins/L (with 95 % confidence intervals of 0.0031 to 0.0057 mg total pyrethrins/L), based on mean measured concentrations, for rainbow trout, 0.010 mg total pyrethrins/L (95 % C.I. of 0.0078 to 0.014 mg/L) based on the mean measured concentrations of total pyrethrins for the Bluegill sunfish, and 0.016 mg total pyrethrins/L (95 % C.I. of 0.014 to 0.018 mg/L) based on the mean measured concentrations of total pyrethrins for the Sheepshead minnow.

The acute toxicity of pyrethrins (Pyrethrum extract (FEK-99) to aquatic invertebrates was tested (Putt A.E., 1994a) in *Daphnia magna* with five test concentrations (mean measured concentrations from 0.0022 to 0.014 mg/L). The test was carried out according to the U.S. EPA Pesticide Assessment Guidelines E, Section 72-2 which is equivalent to the OECD guideline 202. The 48 h EC_{50} for pyrethrins was determined to be 0.012 mg total pyrethrins/L (0.010 – 0.013 mg total pyrethrins/L). OECD TG 202 was also performed with Pyrethrum Extract (FEK-99) and the 6 Pyrethrin esters (Mantilacci, 2015b). In the test, applied concentrations for cinerin 1, pyrethrin 2 and cinerin 2 only reach a maximum of 45 %, whereas for jasmolin 1 and jasmolin 2y, 55 % inhibition was reached. For FEK-99 the EC_{50} = 28.09 µg/L and for pyrethrin 1 EC_{50} = 272.81 µg/L.

Unicellular freshwater green alga, *Desmodesmus subspicatus* was exposed under static conditions for 72 hours to six concentrations of Pyrethrum Extract Pale 50 %/ (Dengler, 2000). This resulted in a NOE_rC = 30.9 mg/L, an E_rC_{50} = 65.1 mg/L, and a E_bC_{50} = 29.0 mg/L (based on nominal concentrations). The total pyrethrum in the test system was above 74 to 91 % of nominal at the beginning and decrease to 61 to 73 % at the end of the test. This resulted in an EC_{50} = 39.8 mg/L and EC_{10} = 19.7 mg/L. Transforming this value to total pyretrins an E_rC_{50} = 19 mg/L and ErC_{10} = 9.85 mg/L was obtained. These values are higher than water solubility and the endpoints were considered as water solubility 0.23 mg/L.

Three acute immobilisation tests with *Chironomus riparius* were performed for the chemical similarity report, with pyrethrum extract 49.35 %, pyrocide 50 % and pyrethrum extract pale 50 % (Dabrunz, A., 2017a, b and c, respectively). They all follow OECD TG 235 "*Chironomus sp.*, Acute Immobilisation Test". In Dabrunz (2017a), observations on immobilization of the *Chironomus riparius* were made after 24 and 48 hours and an EC_{50} of 0.00311 mg total pyrethrins/L based on measured concentration was obtained.

Conclusion

Valid acute toxicity data are presented for fish, invertebrates and algae. In addition, there is data for *Chironomus* based on OECD TG 235 which is a test done in water-only vessels and hence relevant for classification. The use of *Chironomus riparius* values is

further justified by the insecticidal mode of action of the substance. Then, as considered by the DS, RAC notes that *Chironomus riparius* is the most sensitive species with an LC_{50} = 0.00311 mg total pyrethrins/L. Nevertheless, RAC is of the opinion that *H. azteca* LC_{50} from Bradley (2013) is suitable for classification purposes. Then, with the LC_{50} of 0.00076 mg total pyrethrins/L, *H. azteca* is the most sensitive species. Consequently, RAC considers that since the LC_{50} < 1 mg/L, a classification as Aquatic Acute 1 (H400) is warranted and as 0.0001 < LC_{50} ≤ 0.001 mg/L, then an M-factor of 1000 should be applied.

Chronic aquatic toxicity

***Table**: Summary of valid chronic aquatic toxicity tests*

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LOEC/NOEC/EC_{10}[specify the value]		
Fish								
US EPA 72-4 GLP Reliability 2	Fathead minnow (*Pimephales promelas*)	Embryo hatch, survival and growth of larvae	Pyrethrum extract (FEK-99) (Batch R92-254; Purity 57.5 %)	Flow-through	35 days	NOEC 1.9 µg total pyrethrins/L	5 concentrations tested, deaths in all dose groups	A7.4.3.2 (KPIC and BRA, MGK and SCJ)
Invertebrates								
US EPA 72-4/ GLP Reliability 2 Key	*Daphnia magna*	Reproduction /chronic	Pyrethrum extract (FEK-99) (Batch R92-254; Purity 57.5 %)	Flow-through	21 days	NOEC 0.00086 mg total pyrethrins/L	5 concentrations tested, effects observed in the 2 highest concentrations	Putt A.E. (1994b) A7.4.3.4 (KPIC and BRA, MGK and SCJ)
Algae[1]								
OECD TG 201 GLP Reliability 2	*Desmodesmus subspicatus*	Growth and biomass inhibition	Pyrethrum extract Pyrethrum Extract Pale 50 % (Batch 99/11-5 B; Purity 50.17 %)	Static	72 h	NOEC 0.23 mg/L (solubility limit)	6 concentrations tested, significant inhibitory effects from 30.9 - 100 mg Pyrethrum Pale xtract/L for biomass and growth rate	Dengler D. (2000) A7.4.1.3 (KPIC)

Valid aquatic chronic tests are available for the three trophic levels. For fish, a flow-through toxicity test was performed with Fathead minnow (*Pimephales promelas*) during an early life stage exposure (equivalent to OECD TG 210) with pyrethrins. The NOEC and LOEC were determined to be 0.0019 mg total pyrethrins/L and 0.0030 mg total pyrethrins/L, respectively, based on the effects observed for percent embryo hatch and larval growth (total length and wet weight).

Toxicity test about effects on reproduction and growth rate was performed with *Daphnia magna* under flow-through conditions (Putt, 1994b) according to the U.S. EPA Pesticide Assessment Guidelines E, Section 72-4 which is equivalent to the OECD TG 211. Survival of adult daphnids was determined on test days 0, 1, 2, 4, 7, 9, 11, 14, 16, 18 and 21. Following 11 days of exposure, the mean percent survival of organisms exposed to all treatment levels was within the range of 80 – 100 %. Control survival averaged 95 %. Measurements of offspring production was made on days 0, 1, 2, 4, 7 and three times per week thereafter through study termination (day 21). An adverse effect on reproduction was noted in the study and is considered as the most sensitive parameter: the NOEC was determined to be 0.00086 mg total pyrethrins/L.

RAC noted that two other studies are presented and are considered as non-valid by the DS. RAC concurs with the DS that Heintze (2001) is not valid due to the loss of pyrethrins in water observed at the end, the recovery of total pyrethrins detected in the overlaying water was ~1 % of initial amount applied, showing a transfer of pyrethrins from water to sediment phase and a subsequent loss from test system by either degradation or volatilization. Thomas and Krueger (2009) includes deviations that render the study invalid as well. However, RAC takes into consideration the results of the chronic toxicity study with *A. bahia* and Pyrethrum Stewardship Blend. Since RAC considers this study valid and acceptable for classification purpose, *A. bahia* is found to be the most sensitive species with the lowest NOEC = 0.00025 mg of pyrethrin/L.

As *Chrysanthemum cinerariaefolium* extract obtained with hydrocarbon solvent is considered not rapidly degradable (NRD) and the NOEC is < 0.1 mg/L classification as Aquatic Chronic 1 is warranted, the NOEC of 0.00025 mg of pyrethrin/L is in the range 0.0001 < NOEC ≤ 0.001 and leads to a M-factor of 100.

Overall, RAC disagrees with the DS and concludes that **that classification as Aquatic Acute 1 (H400), M = 1000, Aquatic Chronic 1 (H410), M = 100 is warranted.**

A.4. Assessment of additional hazards

A.4.1. Hazardous to the ozone layer

A.4.1.1. *Short summary and overall relevance of the provided information on ozone layer hazard*

Following the physical and chemical properties and the structure of Pyrethrins it is assumed that degradation and persistence of the active substance mainly depends on reaction with hydroxyl-radicals and the average concentration of hydroxyl-radicals in air. Total OH rate constant was determined to be 281.1508 x 10-12 cm³/molec.*sec., mainly due to addition to olefinic bonds (96%) and hydrogen abstraction (4%). Other mechanisms do not contribute to hydroxyl radical estimations. The total rate of both, OH and ozone constant is very low. Half-life in the troposphere was calculated to be 27.391 min for overall OH rate constant and 29.562 min for ozone rate constant. Following the Atkinson calculation, the chemical half-life for Pyrethrum in the troposphere will be below 1 h. It is therefore concluded that Pyrethrins will not accumulate in air and will only be transported on very short distances.

The photochemical oxidative degradation half-life of Pyrethrin 1 in air was calculated according to the method developed by Atkinson , which is based on the structural activity relationship (QSAR´s), by using the Atmospheric Oxidation Program v 1.91 (AOPWIN-software). These estimations were carried out with respect to the OH radical and ozone reactions, using a 12-hours-day with 300.95 x 10-12 and 96.32 x 10-17 cm3/molecule-sec, respectively. The half-lives for the hydroxyl and ozone reactions in air are estimated to be 25.59 and 17.13 minutes, respectively.

A half-life of 76.8 minutes for Pyrethrin 1 in air has been estimated using a 24-hour-day and assuming an OH radical concentration of 5 x 105 radicals.cm-3 according to AOPWIN version 1.91 and following recommendations of ECHA Guidance on Risk Assessment, Chapter 2.3.6.3 .

Stratospheric ozone depletion can be excluded due to the very short half-life in air (DT_{50} in air = 17.133 min), as a result of gas phase reactions with ozone (O_3).

A.4.1.2. *Comparison with the CLP criteria*

Not hazardous to the ozone layer.

Conclusion on classification and labelling for hazardous to the ozone layer.

No classification and labelling required.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

On the basis of the Atkinson calculation, the DS concluded that the chemical half-life for Pyrethrum in the troposphere will be below 1 h and that pyrethrins will not accumulate in air and will only be transported on very short distances.

The photochemical oxidative degradation half-life of pyrethrin 1 in air was calculated according to the method developed by Atkinson, which is based on the structural activity relationship (QSAR´s), by using the Atmospheric Oxidation Program v 1.91 (AOPWIN-software). The half-lives for the hydroxyl and ozone reactions in air are estimated to be 25.59 and 17.13 minutes, respectively. Then, the DS considered that stratospheric ozone depletion can be excluded due to the very short half-life in air (DT_{50} in air = 17.133 min), as a result of gas phase reactions with ozone (O_3).

Comments received during consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

RAC concluded that according to the short half-life of pyrethrins in air, the substance is not hazardous for the stratospheric ozone layer and agrees with the DS that no classification is warranted **for hazards to the ozone layer.**

A.5. Additional Labelling

No further labelling required.

A.6. Assessment of exclusion criteria, substitution criteria and POP

A.6.1. Exclusion criteria

A.6.1.1. *Assessment of CMR properties*

Criteria (BPR Article 5[1])	Assessment
Active substances which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified as, carcinogen category 1A or 1B	Active substance is not classified and does not meet the criteria to be classified as Carc. Cat. 1A or 1B.
Active substances which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified as, mutagen category 1A or 1B	Active substance is not classified and does not meet the criteria to be classified as Muta. Cat. 1A or 1B.
Active substances which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified as, toxic for reproduction category	Active substance is not classified and does not meet the criteria to be classified as Repr. Cat. 1A or 1B.

1A or 1B	
Conclusion on CMR properties	The exclusion criteria in BPR Article 5(1)a-c are not met.

A.6.1.2. *Assessment of endocrine disrupting properties*

Criteria (BPR Article 5)	Assessment
Active substances which, on the basis of the criteria specified pursuant to the first subparagraph of paragraph 3 are considered as having endocrine-disrupting properties that may cause adverse effects in humans and to the environment.	The assessment should be made in accordance with the scientific criteria set out in Commission Delegated Regulation (EU) 2017/2100 and Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.
Conclusion on ED properties:	ED properties have not been sufficiently investigated. It is not possible to ask for additional assays due to BPR article 90. Thus, eCA cannot conclude if the active substance meets or not ED criteria.

A.6.1.3. *PBT Assessment (following Annex XIII to Regulation (EC) No 1907/2006)*

Assessment of persistence

A persistence assessment based on the criteria for identification of persistent or very persistent substances based on REACH Annex XIII was performed. The assessment was performed for *Chrysanthemum cinerariaefolium* extract from HCS.

Screening

Chrysanthemum cinerariaefolium extract from HCS is not readily biodegradable and, therefore, a full assessment of persistence is required.

The persistence assessment is summarised in the tables below.

P Criteria	Assessment
T1/2 > 60 days in seawater, or	-
T1/2 > 40 days in fresh- or estuarine water, or	Photolytic half-life in water estimated to be 11.8 h, therefore, it can be concluded that *Chrysanthemum cinerariaefolium* extract from HCS does not fulfil the criterion for persistence.
T1/2 > 180 days in seawater sediment, or	-
T1/2 > 120 days in freshwater- or estuarine sediment, or	DT_{50} for biodegradation in sediment is 10 d (at 12ºC) (Pyrethrin 1) and 5.27 d at 20ºC, therefore, it can be concluded that *Chrysanthemum cinerariaefolium* extract from HCS does not fulfil the criterion for persistence.
T1/2 <= 120 days in soil.	DT_{50} for biodegradation in soil 3.3 d (at 20ºC) (Pyrethrin

	1), therefore, it can be concluded that *Chrysanthemum cinerariaefolium* extract from HCS does not fulfil the criterion for persistence.

vP Criteria	Assessment
T1/2 > 60 days in sea-, fresh- or estuarine water, or	The vP criterion criteria not fulfilled *Chrysanthemum cinerariaefolium* extract from HCS.
T1/2 > 180 days in seawater-, freshwater- or estuarine sediment, or	
T1/2 > 180 days in soil.	
Conclusion on P / vP properties	*Chrysanthemum cinerariaefolium* extract from HCS does not degrade in a ready biodegradability test and is not assumed to degrade in sewage treatment plants. Pyrethrins are hydrolytically stable to environmental relevant pH but the photolysis is rapid according to laboratory experiments in water that show a photolytic half-life of 11.8 hours. Apart from this, *Chrysanthemum cinerariaefolium* extract from HCS has potential for rapid association to the sediment and consequent degradation according to the estimated half-life in water-sediment system (DT_{50} = 5.27 days at 20°C). Pyrethrins are rapidly photolysed when they are exposed to natural sunlight on the surface of soil (DT_{50} = 12.9 hours) and they are relatively quickly degraded in soil under aerobic conditions (DT_{50} = 3.3 days). Thus, the criterion for persistence established by Table 11-2 in the PBT assessment guidance from ECHA (*Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment*, Version 3.0, June 2017[28]) is not fulfilled.

Assessment of bioaccumulation

An assessment of bioaccumulation potential was performed based on the criteria for identification of bioaccumulative or very bioaccumulative substances based on REACH Annex XIII criteria. The assessment was performed for *Chrysanthemum cinerariaefolium* extract from HCS.

Screening

A substance is considered to have the potential to fulfil the criterion of bioaccumulation when the log Kow exceeds 4.5 (according to the PBT/vPvB Guidance Document[29]).

The log Kow for *Chrysanthemum cinerariaefolium* extract from HCS is 5.59 and hence further assessment of the bioaccumulation potential of *Chrysanthemum cinerariaefolium* extract from HCS extract is required.

The bioaccumulative assessment is summarised in the tables below.

[28] https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf
[29] ECHA (2014); Guidance on Information Requirements and Chemical Safety Assessment; Chapter R.11: PBT/vPvB assessment; Version 2.0; November 2014

B Criteria	Assessment
BCF > 2000	The BCF for fish is 500 which is well below the threshold of 2000.
vB Criteria	**Assessment**
BCF > 5000	-
Conclusion on B/vB properties	*Chrysanthemum cinerariaefolium* extract from HCS has a log Kow equal to 5.59 which is higher than the limit value of 4.5 indicated in the ECHA Guidance. Therefore, this substance may be considered potentially bioaccumulating. Nevertheless, a bioconcentration study of Pyrethrins in fish was submitted by the applicant. The BCF whole body value for Pyrethrins estimated in this study was 500, in addition, a fast depuration rate was estimated (DT_{50}, $_{depuration}$ = 1 day). Based on this result, Pyrethrins do not fulfil the bioaccumulation criterion established in Table 11-2 in the PBT assessment guidance from ECHA (*Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment*, Version 3.0, June 2017[30]) is not fulfilled.

Assessment of toxicity

An assessment of toxicity was performed based on the criteria for identification of toxic substances based on REACH Annex XIII criteria. The assessment was performed for *Chrysanthemum cinerariaefolium* extract from HCS.

Screening

According to the most sensitive endpoint available for *Chrysanthemum cinerariaefolium* extract from HCS from a 21 day *Daphnia magna* study (NOEC 0.86 µg ai/L) the toxic criterion is fulfilled according to the PBT/vPvB Guidance Document.

The toxicity assessment is summarised in the tables below.

T Criteria	Assessment
NOEC/EC_{10} (long-term) < 0.01 mg/L for freshwater or seawater organisms, or	The NOEC for daphnia magna (21 days) is 0.86 µg ai/L which fulfils the criterion for toxicity.
substance meets the criteria for classification as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2) according to the CLP Regulation, or	*Chrysanthemum cinerariaefolium* extract from HCS is not classified as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2) according to the CLP Regulation.

[30] https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf

there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification: specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to the CLP Regulation.	*Chrysanthemum cinerariaefolium* extract from HCS is not classified for specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to the CLP Regulation.
Conclusion on T properties	The toxicity criterion is fulfilled by *Chrysanthemum cinerariaefolium* extract from HCS as the chronic NOEC for aquatic organisms is lower than 0.01 mg/L [limit value established Table 11-2 in the PBT assessment guidance from ECHA (*Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment*, Version 3.0, June 2017[31]). On the other hand, toxicity criteria for mammals are not fulfilled by *Chrysanthemum cinerariaefolium* extract from HCS because of it is not classified as carcinogenic, mutagenic, or toxic for reproduction.

Summary and overall conclusions on PBT or vPvB properties

Overall conclusion:

Chrysanthemum cinerariaefolium extract from HCS cannot be considered as persistent or bioaccumulating. The only criterion fulfilled is Toxicity as it is very toxic to aquatic organisms. Consistently, *Chrysanthemum cinerariaefolium* extract from HCS is not PBT or vPvB.

A.6.2. Substitution criteria

Substitution criteria (BPR, Article 10)	Assessment
One of the exclusion criteria listed in Article 5(1) is met but AS may be approved in accordance with Article 5(2)	Not met.
The criteria to be classified, in accordance	Not met.

[31] https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf

with Regulation (EC) No 1272/2008, as a respiratory sensitiser are met	
The acceptable daily intake, acute reference dose or acceptable operator exposure level, as appropriate, is significantly lower than those of the majority of approved active substances for the same product-type and use scenario	Not met.
Two of the criteria for being PBT in accordance with Annex XIII to Regulation (EC) No 1907/2006 are met	Not met.
There are reasons for concern linked to the nature of the critical effects which, in combination with the use patterns, amount to use that could still cause concern, such as high potential of risk to groundwater, even with very restrictive risk management measures	Not met.
The AS contains a significant proportion of non-active isomers or impurities.	Not met.
Conclusion on substitution criteria	The substitution criteria in BPR Article 10(1)a-f are not met.

A.6.3. Assessment of long-range environmental transportation and impact on environmental compartments

	Assessment
The active substance or a degradation product is a persistent organic pollutant (POP) listed in Annex I of EC 850/2004	The active substance is not listed in Annex I of EC 850/2004.
Assessment of long-range transport potential (LRTAP): Vapour pressure <1000 Pa and half-life in air > 2 days or Monitoring data in remote area showing that the substance is found in remote regions or Result of multimedia modelling	The vapour pressure of Pyrethrin 1, as a representative member of Pyrethrins, is 6.9E-05 Pa (25°C), its half-life in air is of 76.8 minutes (OH radicals) and 17.13 minutes (O_3), indicating that the criterion for long-range transboundary atmospheric transport potential is not fulfilled.
The active substance or a degradation product is vP/vB or T?	The half-life of Pyrethrins in water is lower than two months, in sediment and soil the half-life is lower than 6 months. Therefore, IT cannot be considered as a persistent

	substance. A log Kow equal to 5.59 and a $BCF_{whole\ body}$ of 500 was reported from a bioconcentration study of Pyrethrins in fish, where a fast depuration rate was observed ($DT_{50,\ depuration}$ = 1 day). Thus, the criterion establish for bioaccumulating substances is not fulfil by Pyrethrins. Pyrethrins are very toxic to aquatic organisms (the most sensitive species was reported to be *D. magna*). Toxicity criteria for mammals are not fulfilled as it is not classified as carcinogenic, mutagenic, or toxic for reproduction.
Conclusion on LRTAP/POP assessment	*Chrysanthemum cinerariaefolium* extract from HCS cannot be classified as a POP according to the Executive Body Decision 1998/2 on information to be submitted and the procedure for adding substances to annexes i, ii or iii to the protocol on persistent organic pollutants.

B. Appendices

APPENDIX V: OVERALL REFERENCE LIST (INCLUDING DATA OWNER AND CONFIDENTIALITY CLAIM)

Section A

Author(s)	Section point/ reference number	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data protec-tion claimed yes/no	Owner
Anonymous	A3.2/02	1900	DETERMINATION OF VAPOUR PRESSURE IN REFINED PYRETHRUM EXTRACT Pyrethrum Board of Kenya, Nakuru, Kenya Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK
Anonymous	A3.1.3/01	2000	SPECIFICATION PALE PYRETHRUM EXTRACT 50% Kenya Pyrethrum Information Centre, Oberalm, Austria Kenya Pyrethrum Information Centre Report-no. not applicable GLP: no Published: no	yes	PBK
Anonymous	A6.12.2/01	2001	HUMAN EXPOSURES TO CONSUMER PRODUCTS CONTAINING PYRETHRINS AND PYRETHROIDS: REPORTS TO THE AMERICAN ASSOCIATION OF POISON CONTROL CENTERS 1994-1999 PEGUS Research, Inc., Salt Lake City, UT 84106 Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK
Anonymous	A3.14/01	2006	MATERIAL SAFETY DATA SHEET - PYRETHRUM EXTRACT PALE 50% Kenya Pyrethrum Board, Nakuru, Kenya Kenya Pyrethrum Information Centre Report-no. not applicable GLP: no Published: no Submitted in: A3.3/01	yes	PBK
Anonymous	A3.3/01	2006	MATERIAL SAFETY DATA SHEET - PYRETHRUM EXTRACT PALE 50% Kenya Pyrethrum Board, Nakuru, Kenya Kenya Pyrethrum Information Centre Report-no. not applicable GLP: no Published: no	yes	PBK
Anonymous	A3.8/01	2006	MATERIAL SAFETY DATA SHEET -	yes	PBK

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			PYRETHRUM EXTRACT PALE 50% Kenya Pyrethrum Board, Nakuru, Kenya Kenya Pyrethrum Information Centre Report-no. not applicable GLP: no Published: no **Submitted in: A3.3/01**		
Anonymous	A6.1.4/02	1991	PRIMARY EYE IRRITATION - RABBITS Biosearch Incorporated, Philadelphia, PA 19134 Kenya Pyrethrum Information Centre Report-no. 91-7316A GLP: yes Published: no	yes	PBK
Bienert, U. & Groer, M.	A7.5.1.2/01	1990	ACUTE TOXICITY (14 DAYS) OF PYRETHRUM EXTRAKT TO EARTHWORMS EISENIA FOETIDA (SAVIGNY 1826) IN ARTIFICIAL SOIL IBACON, Rossdorf, Germany Kenya Pyrethrum Information Centre Report-no. 640021 GLP: yes Published: no	yes	PBK
Bruske, L.J.	A3.4/04	1987	REPORT OF ANALYTICAL SERVICES, PYRETHRUM EXTRACT MASS SPECTROMETRY Shrader Lab. Inc., Vinewood, Detroit, Michigan, USA Kenya Pyrethrum Information Centre Report-no. 15335 GLP: no Published: no	yes	PBK
Casida, J.E.	A3.4/03	1973	PYRETHRUM - THE NATURAL INSECTICIDE Academic Press, New York and London Report-no. not applicable GLP: no Published: yes	yes	-
Casida, J.E. , Quistad, G.B.	A6.12.3/01	1995	PYRETHRUM - A BENEFIT TO HUMAN WELFARE Oxford University Press, New York, Oxford Academic Press Report-no. not applicable GLP: no Published: yes	no	-
Casida, J.E., Quistad, G.B.	A5.4/01	1995	PYRETHRUM FLOWERS: PRODUCTION, CHEMISTRY, TOXICOLOGY AND USES -	no	-

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			Oxford University Press, 1995, 217-233 Report-no. not applicable GLP: no Published: yes		
Casida, J.E., Quistad, G.B.	A5.7/01	1995	PYRETHRUM FLOWERS: PRODUCTION, CHEMISTRY, TOXICOLOGY AND USES - Oxford University Press, 1995, 217-233 Report-no. not applicable GLP: no Published: yes Submitted in: A5.4/01	no	-
Comb, T.	A1.3	2021	PYRETHRINS: APPEARANCE AgroChemex Environmental Ltd, Aldhams Farm Research Station, Dead Lane, Manningtree, Essex, CO11 2NF United Kingdom Report ACE-21-459 GLP: yes Published: no	yes	MGK
Comb, T.	A1.3	2021	PYRETHRINS: RELATIVE DENSITY AgroChemex Environmental Ltd, Aldhams Farm Research Station, Dead Lane, Manningtree, Essex, CO11 2NF United Kingdom Report ACE-21-460 GLP: yes Published: no	yes	MGK
Comb, T.	A1.3	2021	PYRETHRINS: SURFACE TENSION AgroChemex Environmental Ltd, Aldhams Farm Research Station, Dead Lane, Manningtree, Essex, CO11 2NF United Kingdom Report ACE-21-461 GLP: yes Published: no	yes	MGK
Comb, T.	A1.3	2021	PYRETHRINS: VISCOSITY AgroChemex Environmental Ltd, Aldhams Farm Research Station, Dead Lane, Manningtree, Essex, CO11 2NF United Kingdom	yes	MGK

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			Report ACE-21-462 GLP: yes Published: no		
Curren, R.D.	A6.6	1989	Unscheduled DNA synthesis assay in rat primary hepatocytes with a confirmatory assay, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850, USA. report no. T8729.380009 GLP: Yes Published: No	Yes	BRA MGK SCJ
Curry, P.T.	A6.6.2/01	1996	CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS Microbiol. Associates Inc., Rockville, Maryland, USA Kenya Pyrethrum Information Centre Report-no. G96AC14.330001 GLP: yes Published: no	yes	PBK
Dabrunz, A.	A7.4.3.5.1	2017a	PY-T-50 Pale Refined Pyrethrins: Toxicity to the larvae of *Chironomus riparius* under Laboratory Conditions (Acute Immobilisation Test – Semi-Static). Rep. N° S16-07030 GLP: yes Published: no	Yes	BRA KPIC MGK
Dabrunz, A.	A7.4.3.5.1	2017b	Pyrocide®50%: Toxicity to the larvae of *Chironomus riparius* under Laboratory Conditions (Acute Immobilisation Test – Semi-Static). Rep.N° S17-00132 GLP: yes Published: no	Yes	BRA KPIC MGK
Dabrunz, A.	A7.4.3.5.1	2017c	Pyrethrum Extract Pale 50%: Toxicity to the larvae of *Chironomus riparius* under Laboratory Conditions (Acute Immobilisation Test – Semi-Static). Rep.N° S17-00133 GLP: yes Published: no	Yes	BRA KPIC MGK
Anonymous	A6.1.5	2017a	Local Lymph Node Assay in Mice (LLNA), MB Research Laboratories, 1765 Wentz Road, P.O. Box 178, Spinnerstown, PA 18968, US, Project No. MB 17-24906.26, Protocol No.5650A-08, 20 Apr 2018 GLP: yes Published: no	Yes	KPIC

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
Anonymous	A6.1.5	2017b	Local Lymph Node Assay in Mice (LLNA), MB Research Laboratories, 1765 Wentz Road, P.O. Box 178, Spinnerstown, PA 18968, US, Project No. MB 17-24904.26, Protocol No. 5650A-08, 20 Apr 2018 GLP: yes Published: no	Yes	MGK
Anonymous	A6.1.5	2017c	Local Lymph Node Assay in Mice (LLNA), MB Research Laboratories, 1765 Wentz Road, P.O. Box 178, Spinnerstown, PA 18968, US, Project No. MB 17-24905.26, Protocol No. 5650A-08, 20 Apr 2018 GLP: yes Published: no	Yes	BRA
Dengler, D.	A7.4.1.3	2000	TESTING OF TOXIC EFFECTS OF PYRETHRUM EXTRACT PALE 50 % ON THE SINGLE CELL GREEN ALGA DESMODESMUS SUBSPICATUS ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Kenya Pyrethrum Information Centre Report-no. 20001028/01-AADs GLP: yes Published: no	yes	PBK
Dengler, D.	A7.4.1.4	2003	ACUTE TOXICITY TESTING OF PYRETHRUM PALE EXTRACT ON ACTIVATED SLUDGE WITH THE RESPIRATION INHIBITION TEST ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Kenya Pyrethrum Information Centre Report-no. 20031289/01-AAHT GLP: yes Published: no	yes	PBK
Donath, C.	A6.6.2	2016a	*In Vitro* Mammalian Micronucleus Assay in Chinese Hamster V79 Cells with Pyrethrum Extreact 50%, Eurofins Biopharma Product Testing Munich GmbH, Behringstrasse 6/8, D-82152 Planegg/Munich, Germany, Study number 164165.GLP: yes Published: no	yes	KPIC
Donath, C.	A6.6.2	2016b	*In vitro* Mammalian Micronucleus Assay in Chinese Hamster V79 Cells with Pyrocide 50%, Eurofins Biopharma Product Testing Munich GmbH, Behringstrasse 6/8, D-82152 Planegg/Munich, Germany, study number 164338.	yes	MGK

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			GLP: yes Published: no		
Donath, C.	A6.6.2	2016c	*In vitro* Mammalian Micronucleus Assay in Chinese Hamster V79 Cells with PY-T-50 Pale Refined Pyrethrins, Eurofins Biopharma Product Testing Munich GmbH, Behringstrasse 6/8, D-82152 Planegg/Munich, Germany, study number 164099. GLP: yes Published: no	yes	BRA
Driz, M.T.	A4.2/01	1994	ANALYTICAL METHOD FOR THE DETERMINATION OF PYRETHRIN BY GAS CHROMATOGRAPHY AND PIPERONYL BUTOXIDE BY LIQUID CHROMATOGRAPHY IN SOIL, SEDIMENT AND WATER Pharmaco LSR Int. INC., East Millstone, NJ 08875 Kenya Pyrethrum Information Centre Report-no. BD-047-92 GLP: yes Published: no	yes	PBK
A. P. Fifi	A7.2.2.1	2015a	Pyrethrum extract components – degradation study in loam soil according to OECD 307. BioTecnologie B. T. Srl, c/o Parco Tecnologico Agroalimentare dell'Umbria, Frazione Pantalla, 06059 Todi (PG), Italy GLP, unpublished	Yes	MGK, BRA, KPIC
A. P. Fifi	A7.2.2.1	2015b	Pyrethrum extract components – degradation study in sandy loam soil according to OECD 307. BioTecnologie B. T. Srl, c/o Parco Tecnologico Agroalimentare dell'Umbria, Frazione Pantalla, 06059 Todi (PG), Italy GLP, unpublished	Yes	MGK, BRA, KPIC
A. P. Fifi	A7.2.2.1	2015c	Pyrethrum extract components – degradation study in loamy sand soil according to OECD 307. BioTecnologie B. T. Srl, c/o Parco Tecnologico Agroalimentare dell'Umbria, Frazione Pantalla, 06059 Todi (PG), Italy GLP, unpublished	Yes	MGK, BRA, KPIC
Anonymous	A6.10/01	2002	DEFINITIVE MECHANISTIC TOXICITY STUDY IN RATS WITH PYRETHRINS Inveresk Research International, Tranent, Scotland	yes	PBK

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			Kenya Pyrethrum Information Centre Report-no. 21029 GLP: yes Published: no		
Anonymous	A6.1.1/01	1991a	ACUTE ORAL TOXICITY, LD50 - RATS Biosearch Incorporated, Philadelphia, PA 19134 Kenya Pyrethrum Information Centre Report-no. 91-7316A GLP: yes Published: no	yes	PBK
Anonymous	A6.1.2/01	1991b	ACUTE DERMAL TOXICITY, SINGLE LEVEL - RABBITS Biosearch Incorporated, Philadelphia, PA 19134 Kenya Pyrethrum Information Centre Report-no. ITPY0027 GLP: yes Published: no	yes	PBK
Anonymous	A6.3.1/01	1987a	TWO WEEK DIETARY EXPLORATORY TOXICITY STUDY IN RATS International Research and Development Corp., Michigan, USA Kenya Pyrethrum Information Centre Report-no. 556-012 GLP: yes Published: no	yes	PBK
Anonymous	A6.3.1/02	1987b	2 WEEK-DIETARY TOXICITY STUDY IN MICE International Research and Development Corp., Michigan, USA Kenya Pyrethrum Information Centre Report-no. 556-014 GLP: yes Published: no	yes	PBK
Anonymous	A6.4.1/01	1988a	EVALUATION OF PYRETHRUM EXTRACT IN A 13-WEEK DOSE RANGE FINDING STUDY IN RATS Int. Res.and Develop.Corp. Mattawan, Michigan 49071, USA Kenya Pyrethrum Information Centre Report-no. 556-010 GLP: yes Published: no	yes	PBK
Anonymous	A6.4.1/02	1988b	EVALUATION OF PYRETHRUM EXTRACT IN A 13-WEEK DOSE RANGE FINDING STUDY IN MICE Int. Res.and Develop.Corp. Mattawan, Michigan 49071, USA Kenya Pyrethrum Information Centre Report-no. 556-008	yes	PBK

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			GLP: yes Published: no		
Anonymous	A6.3.1/03	1988c	EVALUATION OF PYRETHRUM EXTRACT IN AN EIGHT-WEEK DOSE RANGE FINDING TOXICITY STUDY IN DOGS Int. Res.and Develop.Corp. Mattawan, Michigan 49071, USA Kenya Pyrethrum Information Centre Report-no. 556-006 GLP: yes Published: no	yes	PBK
Anonymous	A6.4.1/03	1990a	EVALUATION OF PYRETHRUM EXTRACT IN A ONE YEAR CHRONIC TOXICITY STUDY IN DOGS Int. Res.and Develop.Corp. Mattawan, Michigan 49071, USA Kenya Pyrethrum Information Centre Report-no. 556-007 GLP: yes Published: no	yes	PBK
Anonymous	A6.7/01	1990b	EVALUATION OF PYRETHRUM EXTRACT IN TWO-YEAR DIETARY TOXICITY AND ONCOGENICITY STUDY IN RATS Int. Res.and Develop.Corp. Mattawan, Michigan 49071, USA Kenya Pyrethrum Information Centre Report-no. 556-011 GLP: yes Published: no	yes	PBK
Anonymous	A6.7/02	1990c	EVALUATION OF PYRETHRUM EXTRACT IN AN EIGHTEEN MONTH DIETARY ONCOGENICITY STUDY IN MICE Int. Res.and Develop.Corp. Mattawan, Michigan 49071, USA Kenya Pyrethrum Information Centre Report-no. 556-013 GLP: yes Published: no	yes	PBK
Anonymous	A6.3.2/01	1992	21-DAY REPEATED DOSE DERMAL TOXICITY STUDY WITH PYRETHRUM EXTRACT IN RABBITS Int. Res.and Develop.Corp. Mattawan, Michigan 49071, USA Kenya Pyrethrum Information Centre Report-no. 556-018 GLP: yes Published: no	yes	PBK
Anonymous	A6.12.1/01	1976	HEALTH OF MEN ON LONG-TERM	yes	PBK

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			EXPOSURE TO PYRETHRINS The Pyrethrum Marketing Board, Nakuru, Kenya, East Africa Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no		
Gonsior, G.	A 7.4.3.5.1/ 02	2009	ASSESSMENT OF SIDE EFFECTS OF PYRETHRUM PALE EXTRACT ON THE LARVAE OF THE MIDGE, CHIRONOMUS RIPARIUS WITH THE LABORATORY TEST METHOD eurofins-GAB GmbH, Niefern-Öschelbronn, Germany DKSH Switzerland Ltd. Report-no. S09-00063 GLP: yes Published: no	yes	DKS
Hattermann, D.R.	A7.2.2.2/01	1992a	FIELD PHASE FOR PYRENONE CROP SPRAY (PYRETHRUM + PIPERONYL BUTOXIDE) FIELD DISSIPATION - TERRESTRIAL STUDY APPLIED TO BAREGROUND IN CALIFORNIA, GEORGIA, AND MICHIGAN - VOL.1 Bio/Dynamics, Inc., New Jersey, USA Kenya Pyrethrum Information Centre Report-no. 91189 GLP: yes Published: no	yes	PBK
Hattermann, D.R.	A7.2.2.2/02	1992b	PYRETHRUM ANALYTICAL PHASE FOR PYRENONE CROP SPRAY (PYRETHRUM + PIPERONYL BUTOXIDE) FIELD DISSIPATION - TERRESTERIAL STUDY APPLIED TO BAREGROUND IN CALIFORNIA, GEORGIA, AND MICHIGAN - VOL.2 Bio/Dynamics, Inc., New Jersey, USA Kenya Pyrethrum Information Centre Report-no. 91189 GLP: yes Published: no	yes	PBK
W. Hein	A7.2.1		Degradation of [Cyclopentenone-2-^{14}C]Pyrethrin I in Mußbach soil incubated under aerobic conditions at 20 °C in the dark. RLP AgroScience GmbH, Institut für Agrarökologie, Breitenweg 71, 67435 Neustadt, Germany GLP, unpublished	Yes	MGK, BRA, KPIC
W. Hein, M. Mömdel	A7.1.2.2	2017	[Cyclopentenone-2-^{14}C]pyrethrin 1 "aerobic degradation in natural	Yes	MGK, BRA,

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			water". RLP AgroScience GmbH, Breitenweg 71, 67435 Neustadt, Germany GLP, unpublished		KPIC
Heintze, A.	A7.4.3.5.1	2001	ASSESSMENT OF SIDE EFFECTS OF PYRETHRUM EXTRACT PALE 50 % ON THE LARVAE OF THE MIDGE, CHIRONOMUS RIPARIUS WITH THE LABORATORY TEST METHOD ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Kenya Pyrethrum Information Centre Report-no. 20001028/01-ASCr GLP: yes Published: no	yes	PBK
Anonymous	A6.9/01	1993	ACUTE ORAL NEUROTOXICITY STUDY WITH PYRETHRUM EXTRACT IN RATS Bushy Run Research Center (BRRC) Kenya Pyrethrum Information Centre Report-no. 92N1036 GLP: yes Published: no	yes	PBK
Anonymous	A6.1.3/01	1991	AN ACUTE INHALATION TOXICITY STUDY OF PYRETHRUM EXTRACT IN THE RAT Bio/Dynamics, Inc., New Jersey, USA Kenya Pyrethrum Information Centre Report-no. 91-8331 GLP: yes Published: no	yes	PBK
Hoffmann, H.	A3.11/01	2000	PYRETHRUM PALE EXTRACT 99/11-5B PALE 50%, DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES A.14 A.15 Aventis Research & Technologies, D-65926 Frankfurt a. M. Kenya Pyrethrum Information Centre Report-no. SI072-00 GLP: yes Published: no	yes	PBK
Hoffmann, H.	A3.15/01	2000	PYRETHRUM PALE EXTRACT 99/11-5B PALE 50%, DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES A.14 A.15 Aventis Research & Technologies, D-65926 Frankfurt a. M. Kenya Pyrethrum Information Centre Report-no. SI072-00 GLP: yes Published: no Submitted in: A3.11/01	yes	PBK

ES *Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
Anonymous	A6.6.4/01	1976	MICRONUCLEUS TEST ON PYRETHRUM EXTRACT Huntdingdon Centre, England Kenya Pyrethrum Information Centre Report-no. PYR 8/76707 GLP: no Published: no	yes	PBK
Koopmans, M.J.E.	A7.1.1.2.1	1995	DETERMINATION OF 'READY' BIODEGRADABILITY: CARBON DIOXIDE (CO2) EVOLUTION TEST (MODIFIED STURM TEST) WITH PYRETHRUM EXTRAKT Notox B.V, 5231 DD 's-Hertogenbosch, The Netherlands Kenya Pyrethrum Information Centre Report-no. 141964 GLP: yes Published: no	yes	PBK
Anonymous	A6.10/02	2002	AN INVESTIGATION OF SOME HEPATIC ENZYME ACTIVITIES IN LIVER SAMPLES DERIVED FROM INVERESK STUDY 455790: DEFINITIVE MECHANISTIC TOXICITY STUDY IN RATS WITH PYRETHRINS TNO BIBRA International Ltd. Surrey, UK Kenya Pyrethrum Information Centre Report-no. 4024/2/2/2002 GLP: yes Published: no	yes	PBK
Leng, G., Gries, W., Selim, S.	A6.2/06	2006	BIOMARKER OF PYRETHRUM EXPOSURE not applicable Toxicology Lett, 162, 195-201 Report-no. GLP: no Published: yes	no	-
Lynn, S. P. & Hoxter, K.A.	A7.5.4.1	1991	AN ACUTE CONTACT TOXICITY STUDY WITH PYRETHRUM EXTRACT WITH THE HONEY BEE Wildlife International, Ltd., Easton, Maryland, USA Kenya Pyrethrum Information Centre Report-no. 326-104 GLP: yes Published: no	yes	PBK
Anonymous	A7.4.1.1/01	1994a	PYRETHRUM EXTRACT (FEK-99) - A FLOW-THROUGH ACUTE TOXICITY TEST WITH RAINBOW TROUT (ONCORHYNCHUS MYKISS) Spingborn Lab. Inc., Wareham,	yes	PBK

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			Massachusetts 02571, USA Kenya Pyrethrum Information Centre Report-no. 93-11-5084 GLP: yes Published: no		
Anonymous	A7.4.1.1/02	1994b	PYRETHRUM EXTRACT (FEK-99) - A FLOW-THROUGH ACUTE TOXICITY TEST WITH BLUEGILL SUNFISH (LEPOMIS MACROCHIRUS) Spingborn Lab. Inc., Wareham, Massachusetts 02571, USA Kenya Pyrethrum Information Centre Report-no. 93-8-4916 GLP: yes Published: no	yes	PBK
Anonymous	A7.4.1.1/03	1994c	PYRETHRUM EXTRACT (FEK-99) - A FLOW-THROUGH ACUTE TOXICITY TEST WITH SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) Spingborn Lab. Inc., Wareham, Massachusetts 02571, USA Kenya Pyrethrum Information Centre Report-no. 93-12-5081 GLP: yes Published: no	yes	PBK
Anonymous	A7.4.3.2/01	1994d	PYRETHRUM EXTRACT (FEK-99) - THE TOXICITY TO FATHEAD MINNOW (PIMEPHALES PROMELAS) DURING AN EARLY LIFE-STAGE EXPOSURE Spingborn Lab. Inc., Wareham, Massachusetts 02571, USA Kenya Pyrethrum Information Centre Report-no. 93-10-4983 GLP: yes Published: no	yes	PBK
Maciver, D.R.	A5.3.1/05	1964	MOSQUITO COILS PART II. STUDIES ON THE ACTION OF MOSQUITO COIL SMOKE ON MOSQUITOES - Pyrethrum Post, Journal, 7 (3), 1964, 7-9 Report-no. not applicable GLP: no Published: yes	no	-
Mantilacci, S.	A7.4.1.2	2015a	5 pyrethrin metabolites - acute toxicity to daphnids (*daphnia magna*) under static conditions. rep.n°bt039/15. GLP: yes Published: No	Yes	BRA, MGK, KPIC
Mantilacci, S.	A7.4.1.2	2015b	Pyrethrum Extract (FEK-99) and 6	yes	BRA,

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			Pyrethrin esters - Acute toxicity to Daphnids (*Daphnia magna*) under semi-static conditions, Rep.N°BT038/15. GLP: yes Published: No		MGK, KPIC
Mende, P.	A4.2/04	2006	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF PYRETHRUM IN WATER Eurofins-GAB GmbH, Niefern-Öschelbronn Kenya Pyrethrum Information Centre Report-no. 20051407/01-RVW GLP: yes Published: no	yes	PBK
Mende, P.	A4.2/05	2006	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF PYRETHRUM IN SOIL Eurofins-GAB GmbH, Niefern-Öschelbronn Kenya Pyrethrum Information Centre Report-no. 20051407/01-RVS GLP: yes Published: no	yes	PBK
Mori V.	A7.1.3	2015	Estimation of soil adsorption coefficient (K_{oc}) of Pyrethrum extract components by HPLC. Research Center Biospheres by Biotecnologie B.T., Parco Tecnologico Padano, Via A. Einstein – Loc. Cascina Codazza 26900 Lodi, Italy GLP, unpublished	yes	MGK, BRA, KPIC
Mori V.	A3.9	2015	Determination of the Partition Coefficient N-Octanol/Water (Log Pow) of Pyrethrum Extract Components By HPLC, Research Cener Biospheres, Via A. Einstein-Loc. Cascina Codazza, 26900 LODI, Italy, Report CPU-004-15, 6 May 2015 GLP: yes Published: no	yes	MGK, BRA, KPIC
Morlock, G.	A4.2/03	2006	VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF PYRETHRUM PALE EXTRACT IN AIR GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Kenya Pyrethrum Information Centre Report-no. 20051407/01-CMLU GLP: yes Published: no	yes	PBK
Anonymous	A6.4.3/01	1992	A SUBCHRONIC (3-MONTH)	yes	PBK

ES *Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			INHALATION TOXICITY STUDY OF PYRETHRUN EXTRACT IN THE RAT VIA WHOLE-BODY EXPOSURES Bio/Dynamics, Inc., New Jersey, USA Kenya Pyrethrum Information Centre Report-no. 91-8335 GLP: yes Published: no		
Ochieng, C.D.	A3.9/01	1990	DETERMINATION OF PARTITION COEFFICIENT OF PYRETHRINS IN N-OCTANOL/WATER MIXTURE Pyrethrum Board of Kenya, Nakuru, Kenya Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK
Oellrich, W.	A7.3.1/01	2000	PYRETHRUM EXTRACT, ESTIMATION OF THE PHOTOCHEMICAL OXIDATIVE DEGRADATION GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK
Oellrich, W.	A3.2.1	2001	PYRETHRINS, ANNEX II, POINT 2.3.2 HENRY'S LAW CONSTANT GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK
Oellrich, W.	A3.16/01	2002	PYRETHRINS, ANNEX II, POINT 2.15 OXIDIZING PROPERTIES GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. 104106-029001 GLP: no Published: no	yes	PBK
Oellrich, W.	A3.1.1/01	2003	PYRETHRINS, ANNEX II POINT 2.1.1 MELTING POINT, POINT 2.1.2 BOILING POINT, POINT 2.3.1 VAPOUR PRESSURE DETERMINATION GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. 104108-001-01-rev01 GLP: no	yes	PBK

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			Published: no		
Oellrich, W.	A3.1.2/01	2003	PYRETHRINS, ANNEX II POINT 2.1.1 MELTING POINT, POINT 2.1.2 BOILING POINT, POINT 2.3.1 VAPOUR PRESSURE DETERMINATION GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. 104108-001-01-rev01 GLP: no Published: no **Submitted in: A3.1.1/01**	yes	PBK
Oellrich, W.	A3.2/01	2003	PYRETHRINS, ANNEX II POINT 2.1.1 MELTING POINT, POINT 2.1.2 BOILING POINT, POINT 2.3.1 VAPOUR PRESSURE DETERMINATION GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. 104108-001-01-rev01 GLP: no Published: no Submitted in: A3.1.1/01	yes	PBK
A. Perboni	A7.1.1.1.1.	2015	Pyrethrum extract components-hydrolysis study in water (according to OECD 111). Research Center Biospheres by Biotecnologie B.T., Parco Tecnologico Padano, Via A. Einstein – Loc. Cascina Codazza 26900 Lodi, Italy GLP, unpublished	Yes	MGK, BRA, KPIC
Pfersich, M.	A3.5/01	2001	SOLUBILITY OF PURIFIED ACTIVE SUBSTANCE IN WATER - Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK
Putman, D.L., Morris, M.J.	A6.6.2/02	1989	Chromosome aberrations in Chinese Hamster Ovary (CHO) cells Microbiol. Associates Inc., Rockville, Maryland, USA Kenya Pyrethrum Information Centre Report-no. T8729.337 GLP, Unpublished	yes	PBK
Putt, A.E.	A7.4.1.2	1994a	PYRETHRUM EXTRACT (FEK-99) - ACUTE TOXICITY TO DAPHNIDS (DAPHNIA MAGNA) UNDER FLOW-THROUGH CONDITIONS Spingborn Lab. Inc., Wareham, Massachusetts 02571, USA	yes	PBK

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			Kenya Pyrethrum Information Centre Report-no. 93-11-5082 GLP: yes Published: no		
Putt, A.E.	A7.4.3.4	1994b	PYRETHRUM EXTRACT (FEK-99) - THE CHRONIC TOXICITY TO DAPHNIA MAGNA UNDER FLOW-THROUGH CONDITIONS Spingborn Lab. Inc., Wareham, Massachusetts 02571, USA Kenya Pyrethrum Information Centre Report-no. 94-1-5116 GLP: yes Published: no	yes	PBK
Reynolds, J.L., Robinson, R.A.	A7.1.3	1994	ADSORPTION AND DESORPTION OF [14C]PYRETHRIN 1 IN FOUR SOILS XenoBiotic Laboratories, Inc., Plainsboro, NJ 08536, USA Kenya Pyrethrum Information Centre Report-no. RPT00156 GLP: yes Published: no	yes	PBK
Robinson, R. A.	A7.2.1/01	1994	AEROBIC SOIL METABOLISM OF [14C]PYRETHRIN 1 XenoBiotic Laboratories, Inc., Plainsboro, NJ 08536, USA Kenya Pyrethrum Information Centre Report-no. RPT00204 GLP: yes Published: no	yes	PBK
Robinson, R. A.; Wisocky, M.J.	A7.1.2.2.2/01	1994	AEROBIC AQUATIC METABOLISM OF[14C]PYRETHRIN 1 XenoBiotic Laboratories, Inc., Plainsboro, NJ 08536, USA Kenya Pyrethrum Information Centre Report-no. RPT00193 GLP: yes Published: no	yes	PBK
Anonymous	A6.1.4/01	1991a	PRIMARY SKIN IRRITATION - RABBITS Biosearch Incorporated, Philadelphia, PA 19134 Kenya Pyrethrum Information Centre Report-no. 91-7316A GLP: yes Published: no	yes	PBK
Anonymous	A6.1.5/01	1991b	GUINEA PIG DERMAL SENSITIZATION - MODIFIED BÜHLER METHOD Biosearch Incorporated, Philadelphia, PA 19134 Kenya Pyrethrum Information Centre Report-no. 91-7316A	yes	PBK

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			GLP: yes Published: no		
San, R.H.C., Springfield, K.A.	A6.6.1/01	1989	SALMONELLA/MAMMALIAN-MICROSOME PLATE INCORPORATION MUTAGENICITY ASSAY (AMES TEST) WITH A CONFIRMATORY ASSAY Microbiol. Associates Inc., Rockville, Maryland, USA Kenya Pyrethrum Information Centre Report-no. T8729.501014 GLP: yes Published: no	yes	PBK
Anonymous	A6.8.1/01	1987a	EVALUATION OF PYRETHRUM EXTRACT IN A DEFINITIVE RAT TERATOLOGY STUDY Int. Res.and Develop.Corp. Mattawan, Michigan 49071, USA Kenya Pyrethrum Information Centre Report-no. 556-002 GLP: yes Published: no	yes	PBK
Anonymous	A6.8.1/02	1987b	EVALUATION OF PYRETHRUM EXTRACT IN A DEFINITIVE RABBIT TERATOLOGY STUDY Int. Res.and Develop.Corp. Mattawan, Michigan 49071, USA Kenya Pyrethrum Information Centre Report-no. 556-004 GLP: yes Published: no	yes	PBK
Anonymous	A6.8.2/01	1989	TWO GENERATION REPRODUCTION STUDY IN RATS WITH PYRETHRUM EXTRACT Int. Res.and Develop.Corp. Mattawan, Michigan 49071, USA Kenya Pyrethrum Information Centre Report-no. 556-005 GLP: yes Published: no	yes	PBK
Schmid, J.	A3.4/01	1990a	ANALYTICAL REPORT UV-VIS ABSORPTION SPECTRA OF PYRETRHUM EXTRACT ACCORDING TO OECD GUIDELINE 101 BioChem GmbH, Karlsruhe, Germany Kenya Pyrethrum Information Centre Report-no. GLP: no Published: no	yes	PBK
Schmid, J.	A3.5/02	1990b	WATER SOLUBILITY OF PYRETHRUM EXTRACT ACCORDING TO CIPAC METHOD MT 157.1	yes	PBK

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			BioChem GmbH, Karlsruhe, Germany Kenya Pyrethrum Information Centre Report-no. 947951 GLP: no Published: no		
Schocken, M.J.	A7.4.3.3.1/01	1994	BIOCONCENTRATION STUDY WITH [14C] PYRETHRIN 1 IN BLUEGILL SUNFISH. Spingborn Lab. Inc., Wareham, Massachusetts 02571, USA Kenya Pyrethrum Information Centre Report-no. 94-5-5258 GLP: yes Published: no	yes	PBK
Schreib, G.	A6.6.1	2016a	Reverse Mutation Assay using Bacteria (Salmonella typhimurium and Escherichia coli) with Pyrethrum-Extract 50%, Eurofins Biopharma Product Testing Munich GmbH, Behringstrasse 6/8, 82152 Planegg/Munich, Germany, Report number 164164 GLP: yes Published: no	Yes	KPIC
Schreib, G.	A6.6.1	2016b	Reverse Mutation Assay using Bacteria (*Salmonella typhimurium* and *Escherichia coli*) with Pyrocide 50%, Eurofins Biopharma Product Testing Munich GmBH, Behringstrasse 6/8, 82152 Planegg/Munich, Germany, Report number 164337. GLP: yes Published: no	Yes	MGK
Schreib, G.	A6.6.1	2016c	Reverse Mutation Assay using Bacteria (*Salmonella typhimurium* and *Escherichia coli*) with PY-T-50 Pale Refined Pyrethrins, Eurofins Biopharma Product Testing Munich GmBH, Behringstrasse 6/8, 82152 Planegg/Munich, Germany, Report number 164098. GLP: yes Published: no	Yes	BRA
Schyler, K.C., Massing, R,A,	A5.3.1/04	1974	OBSERVATIONS ON GROUND ULV APPLICTIONS OF SYNERGISED PYRETHRINS ON NON-TARGET INSECTS AND MOSQUITOES IN CENTRE COUNTY, PENNSYLVANIA - Pyrethrum Post, Journal, 12 (4), 1974, 142-144	no	-

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			Report-no. not applicable GLP: no Published: yes		
Anonymous	A6.2/03	2004	A Single Dose, Open Label Study to Investigate the Absorption and Excretion of Orally Administered or Dermally Applied (14C-Labeled Pyrethrin I (PI)) to Healthy Male Volunteers. Pyrethrin Joint Venture Consumer Specialty Product Associations, Inc. 900 17th Street, N.W. Washington, D.C. 20006 Study No.: Sel 0204 GLP Published: No	Yes	MGK
Selim, S.	A7.3.2/01	1993	LABORATORY VOLATILITY OF PYRETHRIN 1 FROM SOIL Biological Test Center, Irvine, CA 92713-9791-USA Kenya Pyrethrum Information Centre Report-no. P0693011 GLP: yes Published: no	yes	PBK
Selim, S.	A7.1.1.1.1	1995a	HYDROLYSIS OF PYRETHRIN 1 AS A FUNCTION OF PH AT 25°C Biological Test Center, Irvine, CA 92713-9791-USA Kenya Pyrethrum Information Centre Report-no. P1092011 GLP: yes Published: no	yes	PBK
Anonymous	A6.2/01	1995b	PHARMACOKINETICS AND METABOLISM OF PYRETHRIN 1 IN THE RAT Biological Test Center, Irvine, CA 92713-9791-USA Kenya Pyrethrum Information Centre Report-no. P1092006 GLP: yes Published: no	yes	PBK
Selim, S.	A7.1.1.1.2/01	1995b	AQUEOUS PHOTOLYSIS OF PYRETHRIN 1 Biological Test Center, Irvine, CA 92713-9791-USA Kenya Pyrethrum Information Centre Report-no. P1192006 GLP: yes Published: no	yes	PBK
Siusiene,E.	A1.4	2022	PHYSICO/CHEMICAL TESTING ON A TEST ITEM ON PYRETHRUM EXTRACT	yes	MGK

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			DEKRA UK Ltd, Phi House, Southampton Science Park, Southampton, SO16 7NS, United Kingdom Report GLP3016010232R1/2021 GLP: yes Pubished: no		
St.Laurent, J.P.	A4.2/02	1995	DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF TOTAL PYRETHRINS IN WATER, ACETONE AND FISH TISSUE Spingborn Lab. Inc., Wareham, Massachusetts 02571, USA Kenya Pyrethrum Information Centre Report-no. 93-9-4922 GLP: yes Published: no	yes	PBK
Steenwinkel, M-J.S.T.	A6.6.3/01	2001	GENE MUTATION TEST AT THE TK-LOCUS OF L5178Y CELLS WITH PYRETHRIN TNO Nutrition and Food Res., Zeist, The Netherlands Kenya Pyrethrum Information Centre Report-no. 2503/06 GLP: yes Published: no	yes	PBK
Straube, D.	A7.5.2.1	2016a	PY-T-50 Pale Refined Pyrethrins; Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5% Peat: ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany: unpublished report no., 114001016 (15 Sep 2016). GLP: yes	yes	BRA, MGK, KPIC
Straube, D.	A7.5.2.1	2016b	PY-T-50 Pale Refined Pyrethrins; Effects on Reproduction of the Predatory Mite Hypoaspis aculeifer in Artificial Soil with 5% Peat: ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany: unpublished report no., 114001089 (15 Sep 2016).	yes	BRA, MGK, KPIC
Straube, D.	A7.5.2.1	2016c	PYROCIDE® 50%: Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5% Peat: ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany: unpublished report no., 110901016	yes	BRA, MGK, KPIC

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			(12 Oct 2016). GLP: yes		
Straube, D.	A7.5.2.1	2016d	*PYROCIDE® 50%: Effects on Reproduction of the Predatory Mite* Hypoaspis aculeifer *in Artificial Soil with 5% Peat: ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany: unpublished report no., 110901089 (12 Oct 2016).*	Yes	BRA, MGK, KPIC
Straube, D.	A7.5.2.1	2016e	Pyrethrum Extract Pale 50%: Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5% Peat: ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany: unpublished report no., 114011016 (15 Sep 2016).	Yes	BRA, MGK, KPIC
Straube, D.	A7.5.2.1	2016f	Pyrethrum Extract Pale 50%: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat: ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany: unpublished report no., 114011089 (16 Sep 2016).	yes	BRA, MGK, KPIC
Sum, K.S., Kimani, S.M., Kuria, J.N.	A5.3.1/03	2005	EMPIRICAL BIO-EFFICACY OF PYRETHRINS AND PYRETHROIDS IN AEROSOL FORMULATIONS FOR MOSQUITO CONTROL - Pyrethrum Post, Journal, 20 (04), 2005, 140-149 Report-no. not applicable GLP: no Published: yes	no	-
Testman, R.	A7.2.2.4/01	1994	SOIL SURFACE PHOTOLYSIS OF PYRETHRIN 1. Biological Test Center, Irvine, CA 92713-9791-USA Kenya Pyrethrum Information Centre Report-no. P1192007 GLP: yes Published: no	yes	PBK
Tiemann, J.	A3.2/03	2004	PYRETHRINS, ANNEX II POINT 2.3.1 VAPOUR PRESSURE OF PURIFIED A.S., POINT 2.3.2 HENRY'S LAW CONSTANT GAB Consulting GmbH, Lamstedt, Germany	yes	PBK

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			Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no		
Troese, M.	A6.1.5	2017a	*In vitro* Sensitization Assay (IVSA) Pyrethrum Extract Pale (50% w/w), MB Research Laboratories, 1765 Wentz Road, P.O.Box 178, Spinnerstown, PA 18968, USA, MB 17-24906.19 {Not signed and dated} GLP: yes Published: no	Yes	KPIC
Troese, M.	A6.1.5	2017b	*In vitro* Sensitization Assay (IVSA) Refined Pyrethrum Concentrate (53.72% w/w), MB Research Laboratories, 1765 Wentz Road, P.O.Box 178, Spinnerstown, PA 18968, USA, MB 17-24904.19 {Not signed and dated} GLP: yes Published: no	Yes	MGK
Troese, M.	A6.1.5	2017c	*In vitro* Sensitization Assay (IVSA) PY-T-50 Pale Refined Pyrethrins (49.36% w/w), MB Research Laboratories, 1765 Wentz Road, P.O.Box 178, Spinnerstown, PA 18968, USA, MB 17-24905.19 {Not signed and dated} GLP: yes Published: no	Yes	BRA
Wachter, S.	A7.5.1.1	2000	ASSESSMENT OF THE SIDE EFFECTS OF PYRETHRUM EXTRACT PALE 50 % ON THE ACTIVITY OF THE SOIL MICROFLORA ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Kenya Pyrethrum Information Centre Report-no. 20001028/01-ABMF GLP: yes Published: no	yes	PBK
Wallner, B.	A6.6.1	2016a	*In vitro* Mammalian Cell Gene Mutation Assay (Thymidine Kinase Locus/$TK^{+/-}$), Eurofins Biopharma Product Testing Munich GmbH, Behringstrasse 6/8, D-82152 Planegg/Munich, Germany, Report number 164166. GLP: yes Published: no	Yes	KPIC
Wallner, B.	A6.6.1	2016b	*In vitro* Mammalian Cell Gene	Yes	MGK

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			Mutation Assay (thymidine Kinase Locus/TK+/-) in Mouse Lymphoma L5178Y Cells with Pyrocide 50% Eurofins Biopharma Product Testing Munich GmbH, Behringstrasse 6/8, D-82152 Planegg/Munich, Germany, Report number 164339 GLP: yes Published: no		
Wallner, B.	A6.6.1	2016c	*In vitro* Mammalian Cell Gene Mutation Assay (thymidine Kinase Locus/TK+/-) in Mouse Lymphoma L5178Y Cells with PY-T-50 Pale Refined Pyrethrins Eurofins Biopharma Product Testing Munich GmbH, Behringstrasse 6/8, D-82152 Planegg/Munich, Germany, Report number 164100 GLP: yes Published: no	Yes	BRA
Walter, D.	A 3.1.3/02	2008a	RELATIVE DENSITY OF "PALE" PYRETHRUM EXTRACT 50 % eurofins-GAB GmbH, Niefern-Öschelbronn, Germany Kenya Pyrethrum Information Centre Report-no. 20071557/01-PCRD GLP: yes Published: no	yes	PBK
Walter, D.	A3.7/01	2000a	SOLUBILITY OF PYRETHRUM EXTRACT PALE 50% IN ORGANIC SOLVENTS ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Kenya Pyrethrum Information Centre Report-no. 20001028/01-PSBO GLP: yes Published: no	yes	PBK
Walter, D.	A3.12/01	2000b	FLASH POINT OF PYRETHRUM EXTRACT PALE 50 % ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Kenya Pyrethrum Information Centre Report-no. 20001028/01-PCFB GLP: yes Published: no	yes	PBK
Walter, D.	A3.13/01	2005	SURFACE TENSION OF PYRETRHUM EXTRACT PALE 50% GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Kenya Pyrethrum Information Centre Report-no. 20051087/01-PCST GLP: yes Published: no	yes	PBK
Walter, D.	A 3.14/02	2008b	VISCOSITY OF "PALE" PYRETHRUM	yes	PBK

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			EXTRACT 50 % eurofins-GAB GmbH, Niefern-Öschelbronn, Germany Kenya Pyrethrum Information Centre Report-no. 20071557/01-PCVC GLP: yes Published: no		
Warui, C.	A5.3.1/01	1996a	TO COMPARE VARIOUS AEROSOL FORMULATIONS IN THE CONTROL OF HOUSEFLIES MUSCA DOMESTICA Kenya Pyrethrum Board, Nakuru, Kenya Kenya Pyrethrum Information Centre Report-no. 896/1136 GLP: no Published: no	yes	PBK
Warui, C.	A5.3.1/02	1996b	TO COMPARE VARIOUS AEROSOL FORMULATIONS IN THECONTROL OF COCKROACHES Kenya Pyrethrum Board, Nakuru, Kenya Kenya Pyrethrum Information Centre Report-no. 896/1136 GLP: no Published: no	yes	PBK
Werle, H.	A7.1.1.1.2/02	1992	PHOTOLYSIS STUDY (QUANTUM YIELD) OF PYRETHRUM IN WATER BioChem GmbH, Karlsruhe, Germany Kenya Pyrethrum Information Centre Report-no. 915040180 GLP: no Published: no	yes	PBK
Werle, H.	A3.4/02	1994	REPORT IR-SPECTRUM PYRETHRUM EXTRACT BioChem GmbH, Karlsruhe, Germany Kenya Pyrethrum Information Centre Report-no. 945040321 GLP: yes Published: no	yes	PBK
Anonymous	A6.2/02	1994	HUMAN IN VIVO PERCUTANEOUS ABSORPTION OF PYRETHRIN AND PIPERONYL BUTOXIDE - Food Chem Toxicol, 32, 51 - 53 Report-no. Not applicable GLP: no Published: yes	no	-
Witte, A.	A7.1.2.2.2/02	2007	DEGRADATION AND METABOLISM OF PYRETHRIN 1 IN TWO WATER/SEDIMENT SYSTEMS UNDER AEROBIC CONDITIONS - LABORATORY	yes	PBK

ES *Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
			TEST eurofins-GAB GmbH, Niefern-Öschelbronn, Germany Pyrethrum Board of Kenya Report-no. 20061275/01-CUWS GLP: yes Published: no		

Section B Product A (AquaPy)

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
Balluff, M.	B7.8.6/01		AQUAPY: A GREENHOUSE TOXICITY STUDY TO DETERMINE THE EFFECTS OF A 30 G AI L-1 PIPERONYL BUTOXIDE EW FORMULATION ON THE VEGETATIVE VIGOUR OF SIX SPECIES OF PLANTS GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20054072/S2-FGVV GLP: no Published: no	yes	BES
Blagden, S.M.	B6.1.3/01	1994	AQUA PYBUTHRIN: ACUTE INHALATION TOXICITY STUDY FOUR HOUR EXPOSURE (NOSE ONLY) IN THE RAT Safepharm Laboratories Limited, Derby, UK Bayer ES Report-no. GR94-0003 GLP: yes Published: no	yes	BES
Bocksch, S.	B7.8.2/01	2005	ASSESSMENT OF SIDE EFFECTS OF AQUAPY ® (PBO+PYR EW 135+30A G) TO THE HONEY BEE, APIS MELLIFERA L., IN THE LABORATORY GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-BLEU GLP: yes Published: no	yes	BES
Boucaud, B.	B6.6/01	2006	OCCUPATIONAL MEDICAL EXPERIENCES WITH PYRETHRE not applicable Bayer ES Report-no. not applicable GLP: no Published: no	yes	BES

Bowron, M.J.	B5.10.2/05	1993	AN EVALUATION OF AQUA PYBUTHRIN IN DRY DEPOSIT AND WET WALKOVER TESTS AGAINST BLATTELLA GERMANICA Roussel Uclaf Environmental Health, Berkhamstedt Bayer ES Report-no. REPE 93-C8 GLP: no Published: no	no	BES
Clouzeau, J.	B6.1.1/01	1993a	ACUTE ORAL TOXICITY IN RATS Centre International de Toxicologie, Evreux, France Bayer ES Report-no. 9965 TAR GLP: yes Published: no	yes	BES
Clouzeau, J.	B6.1.2/01	1993b	ACUTE DERMAL TOXICITY IN RATS Centre International de Toxicologie, Evreux, France Bayer ES Report-no. 9966 TAR GLP: yes Published: no	yes	BES
Clouzeau, J.	B6.2.1/01	1993c	ACUTE DERMAL IRRITATION IN RABBITS Centre International de Toxicologie, Evreux, France Bayer ES Report-no. 9967 TAL GLP: yes Published: no	yes	BES
Clouzeau, J.	B6.2.2/01	1993d	ACUTE EYE IRRITATION IN RABBITS Centre International de Toxicologie, Evreux, France Bayer ES Report-no. 9968 TAL GLP: yes Published: no	yes	BES
Clouzeau, J.	B6.3/01	1993e	SKIN SENSITIZATION TEST IN GUINEA-PIGS Centre International de Toxicologie, Evreux, France Bayer ES Report-no. 9969 TSG GLP: yes Published: no	yes	BES
Dengler, D.	B7.7.1.1/03	2005	TESTING OF TOXIC EFFECTS OF AQUAPY ON THE SINGLE CELL GREEN ALGA PSEUDOKIRCHNERIELA SUBCAPITATA (FORMERLY SELENASTRUM CAPROCORNUTUM GAB Biotechn. GmbH & IFU Umweltanalytik GmbH, Germany Bayer ES Report-no. 20051085/01-AAPs GLP: yes Published: no	yes	BES

Dobrat, W., Martijn, A.	B4.1/01	1998	PYRETHRUM + PIPERONYL BUTOXIDE + MGK 264 TECHNICAL CONCENTRATES 32+33+345/TK/(M)/- - CIPAC Collaborative International Pesticides Analytical Council, H 1998, 239-242 Report-no. not applicable GLP: no Published: yes	no	-
Kölzer, U.	B7.8.4/01	2005a	ACUTE TOXICITY OF AQUAPY ON EARTHWORMS, EISENIA FETIDA USING AN ARTIFICIAL SOIL TEST GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-NLEf GLP: yes Published: no	yes	BES
Kölzer, U.	B7.8.5/01	2005b	ASSESSMENT OF THE SIDE EFFECTS OF AQUAPY® ON THE ACTIVITY OF THE SOIL MICROFLORA GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-ABMF GLP: yes Published: no	yes	BES
Lucas, J.R., Bowron, M.J.	B5.10.2/01	1994a	AN EVALUATION OF THE BIOLOGICAL ACTIVITY OF AQUAPY (TF2578) WHEN APPLIED OUT OF DOORS AS A ULV SPACE SPRAY AGAINST CAGED MUSCA DOMESTICA AND CULEX QUINQUEFASCIATUS Roussel Uclaf Environmental Health, Berkhamstedt Bayer ES Report-no. GB94-0121 GLP: no Published: no	no	BES
Lucas, J.R., Bowron, M.J.	B5.10.2/03	1994b	AN EVALUATION OF THE BIOLOGICAL PERFORMANCE OF AQUAPY WHEN APPLIED IN A 42 M3 CHAMBER AS A MIST AGAINST HOUSEFLIES AND CLOTHES MOTHS Roussel Uclaf Environmental Health, Berkhamstedt Bayer ES Report-no. GB94-0019 GLP: no Published: no	no	BES
Lucas, J.R., Bowron, M.J.	B5.10.2/04	1994c	THERMAL FOGGING OF AQUAPY (TF2578) AGAINST CULEX QUINQUEFASCIATUS Roussel Uclaf Environmental Health, Berkhamstedt Bayer ES Report-no. GB94-0033 GLP: no Published: no	no	BES

Mooney, M.	B6.6/02	1996a	A FIELD EVALUATION OF OPERATOR EXPOSURE TO AQUAPY SPACE SPRAY IN A TOBACCO WAREHOUSE Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer ES Report-no. GD96-0002 GLP: no Published: no	yes	BES
Mooney, M.	B6.6/03	1996b	A FIELD EVALUATION OF THE PHYSICAL CHARACERISTICS OF AQUAPY SPACE SPRAY IN A TOBACCO WAREHOUSE Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer ES Report-no. GD96-0001 GLP: no Published: no	yes	BES
Moretto, A.	B6.5/01	1995	PIPERONYL BUTOXIDE - A MONOGRAPH PREPARED BY THE JOINT FAO/WHO MEETING ON PESTICIDES RESIDUES Istituto di Medicina del Lavoro, Padua, Itlay Report-no. not applicable GLP: no Published: yes	no	-
Ochieng, C.D.	A3.9/01	1990	DETERMINATION OF PARTITION COEFFICIENT OF PYRETHRINS IN N-OCTANOL/WATER MIXTURE Pyrethrum Board of Kenya, Nakuru, Kenya Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK
Oellrich, W.	A7.3.1/01	2000	PYRETHRUM EXTRACT, ESTIMATION OF THE PHOTOCHEMICAL OXIDATIVE DEGRADATION GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK
Oellrich, W.	A3.2.1/01	2001	PYRETHRINS, ANNEX II, POINT 2.3.2 HENRY'S LAW CONSTANT GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK

Oellrich, W.	A3.1.1/01	2003	PYRETHRINS, ANNEX II POINT 2.1.1 MELTING POINT, POINT 2.1.2 BOILING POINT, POINT 2.3.1 VAPOUR PRESSURE DETERMINATION GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. 104108-001-01-rev01 GLP: no Published: no	yes	PBK
Repetto-Larsay, M.	B6.3/02	2005	PBO+PYR EW 135+30A G(AQUAPY) (PIPERONYLBUTOXID 139.86 G/L, PYRETHRUM 33.07 G/L) EVALUATION OF THE POTENTIAL DERMAL SENSITIZATION IN THE LOCAL LYMPH NODE ASSAY IN THE MOUSE Bayer CropScience, Sophia Antipolis, France Bayer ES Report-no. SA 05101 GLP: yes Published: no	yes	BES
Reynolds, J.L., Robinson, R.A.	A7.1.3/01	1994	ADSORPTION AND DESORPTION OF [14C]PYRETHRIN 1 IN FOUR SOILS XenoBiotic Laboratories, Inc., Plainsboro, NJ 08536, USA Kenya Pyrethrum Information Centre Report-no. RPT00156 GLP: yes Published: no	yes	PBK
Robinson, R. A.	A7.2.1/01	1994	AEROBIC SOIL METABOLISM OF [14C]PYRETHRIN 1 XenoBiotic Laboratories, Inc., Plainsboro, NJ 08536, USA Kenya Pyrethrum Information Centre Report-no. RPT00204 GLP: yes Published: no	yes	PBK
Robinson, R. A.; Wisocky, M.J.	A7.1.2.2.2/01	1994	AEROBIC AQUATIC METABOLISM OF[14C]PYRETHRIN 1 XenoBiotic Laboratories, Inc., Plainsboro, NJ 08536, USA Kenya Pyrethrum Information Centre Report-no. RPT00193 GLP: yes Published: no	yes	PBK
Schmid, J.	A3.5/02	1990	WATER SOLUBILITY OF PYRETHRUM EXTRACT ACCORDING TO CIPAC METHOD MT 157.1 BioChem GmbH, Karlsruhe, Germany Kenya Pyrethrum Information Centre Report-no. 947951 GLP: no Published: no	yes	PBK

Selim, S.	A7.1.1.1.1/01	1995a	HYDROLYSIS OF PYRETHRIN 1 AS A FUNCTION OF PH AT 25°C Biological Test Center, Irvine, CA 92713-9791-USA Kenya Pyrethrum Information Centre Report-no. P1092011 GLP: yes Published: no	yes	PBK
Selim, S.	A7.1.1.1.2/01	1995b	AQUEOUS PHOTOLYSIS OF PYRETHRIN 1 Biological Test Center, Irvine, CA 92713-9791-USA Kenya Pyrethrum Information Centre Report-no. P1192006 GLP: yes Published: no	yes	PBK
Stäbler, D.	B7.7.1.1/01	2005a	ACUTE TOXICITY TESTING OF AQUAPY IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) (TELEOSTEI, SALMONIDAE) GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-AAOm GLP: yes Published: no	yes	BES
Stäbler, D.	B7.7.1.1/02	2005b	ASSESSMENT OF TOXIC EFFECTS OF AQUAPY ON DAPHNIA MAGNA USING THE 48 H ACUTE IMMOBILISTAION TEST GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-AADm GLP: yes Published: no	yes	BES
Tolosa, M.	B5.10.2/02	2006	FIELD CONDITIONS EVALUATION OF ADULTICIDE EFFICACY BY TERRESTRIAL APPLICATION OF THE AQUAPY PREPARATION (AQUEOUS EMULSION BASED ON 30 G PYRETHRINS AND 135 G PIPERONYL BUTOXIDE/L)ON MOSQUITO PESTS OCHLEROTATUS CASPIUS, OC. DEDRITUS, AEDES VEXANS (DIPTERA-CULICIDAE) EID Méditerranée Bayer ES Report-no. EID 05049 GLP: no Published: no	yes	BES
Warmers, C.	B7.8.3/01	2005a	AQUAPY ®: TOXICITY TO THE PREDATORY MITE, TYPHLODROMUS PYRI SCHEUTEN (ACARI, PHYTOSEIIDAE) IN THE LABORATORY (RATE RESPONSE TEST) GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-NLTp GLP: yes Published: no	yes	BES

Warmers, C.	B7.8.3/02	2005b	AQUAPY ®: ACUTE TOXICITY TO THE APHID PARASITOID. APHIDIUS RHOPALOSIPHI DE STEFANI PEREZ (HYMENOPTERA, BRACONIDAE) IN THE LABORATORY (RATE RESPONSE TEST) GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-NLAp GLP: yes Published: no	yes	BES

Doc II-B (RAID / Baygon Pyrethrum Mat)

Author(s)	**Section point/ reference number**	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**	**Data protec-tion claimed** **yes/no**	**Owner**
Anonymous	B5.10.2/02	2000	WORK REQUEST REPORT, STANDARD GLASS CHAMBER TEST WITH MOSQUITOES (CULEX QUINQUEFASCIATUS) - SC JOHNSON & SON, INC., RACINE REPORT-NO. W-145904 GLP: NO PUBLISHED: NO	yes	SCJ
Dobrat, W., Martijn, A.	B4.1/01	1998	PYRETHRUM + PIPERONYL BUTOXIDE + MGK 264 TECHNICAL CONCENTRATES 32+33+345/TK/(M)/- - CIPAC Collaborative International Pesticides Analytical Council, H 1998, 239-242 Report-no. not applicable GLP: no Published: yes	no	-
Eberhardt, R., Wieland, K.	B6.6/01	2006	DETERMINATION OF EVAPORATION-KINETICS OF A SHORT TERM VAPORIZER PYRETHRUM MATS FORMULA NO: 63136-002 BioGenius GmbH, Monheim, Germany SC Johnson & Son, Inc., Racine Report-no. Mo 3133 GLP: yes Published: no	yes	SCJ
Görgulü, N.	B4.1/02	2006	VALIDATION OF METHOD MV002: DETERMINATION OF PYRETHRUM IN CELLULOSE MATS BioGenius GmbH, Monheim, Germany SC Johnson & Son, Inc., Racine Report-no. Mo2992 GLP: yes Published: no	yes	SCJ
Kristopeit, K.A.,	B5.10.2/03	1995	WORK REQUEST REPORT	yes	SCJ

Sosa, A., Petersen, J.			NOT APPLICABLE SC JOHNSON & SON, INC., RACINE REPORT-NO. NOT APPLICABLE GLP: NO PUBLISHED: NO		
Moretto, A.	B6.5/01	1995	PIPERONYL BUTOXIDE - A MONOGRAPH PREPARED BY THE JOINT FAO/WHO MEETING ON PESTICIDES RESIDUES Istituto di Medicina del Lavoro, Padua, Itlay Report-no. not applicable GLP: no Published: yes	no	-
Ochieng, C.D.	A3.9/01	1990	DETERMINATION OF PARTITION COEFFICIENT OF PYRETHRINS IN N-OCTANOL/WATER MIXTURE Pyrethrum Board of Kenya, Nakuru, Kenya Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK
Oellrich, W.	A3.2.1/01	2001	PYRETHRINS, ANNEX II, POINT 2.3.2 HENRY'S LAW CONSTANT GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. not stated GLP: no Published: no	yes	PBK
Oellrich, W.	A3.1.1/01	2003	PYRETHRINS, ANNEX II POINT 2.1.1 MELTING POINT, POINT 2.1.2 BOILING POINT, POINT 2.3.1 VAPOUR PRESSURE DETERMINATION GAB Consulting GmbH, Lamstedt, Germany Kenya Pyrethrum Information Centre Report-no. 104108-001-01-rev01 GLP: no Published: no	yes	PBK
Selim, S.	A6.2/01	1995b	PHARMACOKINETICS AND METABOLISM OF PYRETHRIN 1 IN THE RAT Biological Test Center, Irvine, CA 92713-9791-USA Kenya Pyrethrum Information Centre Report-no. P1092006 GLP: yes Published: no	yes	PBK
Verwey, R.E.	B5.10.2/01	2005	WORK REQUEST REPORT, STANDARD GLASS CHAMBER TEST WITH MOSQUITOES (CULEX QUINQUEFASCIATUS) - SC JOHNSON & SON, INC., RACINE REPORT-NO. W-211682 GLP: NO PUBLISHED: NO	yes	SCJ
Wester, R.C., Bucks D.A.W., Maibach H.I.	A6.2/02	1994	HUMAN IN VIVO PERCUTANEOUS ABSORPTION OF PYRETHRIN AND PIPERONYL BUTOXIDE - Food Chem Toxicol, 32, 51 - 53 Report-no. Not applicable	no	-

			GLP: no Published: yes		

Additional references

Andres E, Barry M, Hundt A, et al. A new prediction model for distinguishing skin sensitisers based on IL-18 release from reconstructed epidermis: enhancing the assessment of a key event in the skin sensitisation adverse outcome pathway. J Dermat Cosmetol . 2020;4(5):123-137.

Cariello NF, Piegorsch WW. The Ames test: the two-fold rule revisited. Mutat Res. 1996;369(1-2):23-31.

Cohen SM. Evaluation of possible carcinogenic risk to humans based on liver tumors in rodent assays: the two-year bioassay is no longer necessary. Toxicol Pathol. 2010;38(3):487-501.

Deng W, Oldach J, Armento A, Ayehunie S, Kandarova H, Letasiova S, Klausner M, Hayden P. MatTek Corporation, Ashland, MA, USA. Il-18 secretion as a marker for identification of contact sensitizers in the Epiderm in vitro human skin model. SOT 2011 poster abstract. Toxicologist. 120.

Elcombe CR, Peffer RC, Wolf DC, Bailey J, Bars R, Bell D, Cattley RC, Ferguson SS, Geter D, Goetz A, Goodman JI, Hester S, Jacobs A, Omiecinski CJ, Schoeny R, Xie W, Lake BG. Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit Rev Toxicol. 2014;44(1):64-82.

Finch JM, Osimitz TG, Gabriel KL, Martin T, Henderson WJ, Capen CC, Butler WH, Lake BG. A mode of action for induction of thyroid gland tumors by Pyrethrins in the rat. Toxicol Appl Pharmacol. 2006;214(3):253-62.

Garcia-Bravo B, Rodriguez-Pichardo A, de Pierola SF, Camacho F. Airborne erythema-multiforme-like eruption due to pyrethrum. Contact Dermatitis. 1995 Dec;33(6):433.

Gibbs S, Corsini E, Spiekstra SW, Galbiati V, Fuchs HW, Degeorge G, Troese M, Hayden P, Deng W, Roggen E. An epidermal equivalent assay for identification and ranking potency of contact sensitizers. Toxicol Appl Pharmacol. 2013 Oct 15;272(2):529-41.

Hurley PM. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. Environ Health Perspect. 1998;106(8):437-45

Kaufmann W, Bader R, Ernst H, Harada T, Hardisty J, Kittel B, Kolling A, Pino M, Renne R, Rittinghausen S, Schulte A, Wöhrmann T, Rosenbruch M. 1st international ESTP expert workshop: "Larynx squamous metaplasia". A re-consideration of morphology and diagnostic approaches in rodent studies and its relevance for human risk assessment. Exp Toxicol Pathol. 2009;61(6):591-603.

LeBaron MJ, Gollapudi BB, Terry C, Billington R, Rasoulpour RJ. Human relevance framework

for rodent liver tumors induced by the insecticide sulfoxaflor. Crit Rev Toxicol. 2014;44 Suppl 2:15-24.

Ko GM, Rosenkranz A, Bertoncini CRA, Jurkiewicz NH, Franco MG, Jurkiewicz A. Methods of acute biological assays in guinea-pigs for the study of toxicity and innocuity of drugs and chemicals. Braz J Pharm Sci. 2010;46(2):251-63.

Mecklenburg L, Kusewitt D, Kolly C, Treumann S, Adams ET, Diegel K, Yamate J, Kaufmann W, Müller S, Danilenko D, Bradley A. Proliferative and non-proliferative lesions of the rat and mouse integument. J Toxicol Pathol. 2013;26(3 Suppl):27S-57S.

Mitchell JC, Dupuis G, Towers GH. Allergic contact dermatitis from pyrethrum (Chrysanthemum spp.). The roles of pyrethrosin, a sesquiterpene lactone, and of pyrethrin II. Br J Dermatol. 1972 Jun;86(6):568-73.

Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutat Res. 2000;455(1-2):29-60.

OECD Environment, Health and Safety Publications. Series on Testing & Assessment, No. 256. Guidance document on the reporting of defined approaches and individual information sources to be used within integrated approaches to testing and assessment (IATA) for skin sensitisation. ENV/JM/MONO(2016)29, Paris, 2016a

OECD Environment, Health and Safety Publications. Series on Testing and Assessment, No. 238 - 2nd edition. Overview of the set of OECD Genetic Toxicology Test Guidelines and updates performed in 2014-2015. ENV/JM/MONO(2016)33/REV1, Paris, 2016b

Osimitz TG, Droege W, Finch JM. Toxicologic significance of histologic change in the larynx of the rat following inhalation exposure: a critical review. Toxicol Appl Pharmacol. 2007;225(3):229-37.

Osimitz TG, Lake BG. Mode-of-action analysis for induction of rat liver tumors by pyrethrins: relevance to human cancer risk. Crit Rev Toxicol. 2009;39(6):501-11.

Osimitz TG, Sommers N, Kingston R. Human exposure to insecticide products containing pyrethrins and piperonyl butoxide (2001-2003). Food Chem Toxicol. 2009 Jul;47(7):1406-15.

Price RJ, Giddings AM, Scott MP, Walters DG, Capen CC, Osimitz TG, Lake BG. Effect of Pyrethrins on cytochrome P450 forms in cultured rat and human hepatocytes. Toxicology. 2008;243(1-2):84-95.

Renne RA, Gideon KM, Harbo SJ, Staska LM, Grumbein SL. Upper respiratory tract lesions in inhalation toxicology. Toxicol Pathol. 2007;35(1):163-9.

Sisti A, Huayllani MT, Boczar D, Fosko S, Forte AJ. Resolution of Keratoacanthoma Type Squamous Cell Carcinoma Following Intralesional Therapy With Methotrexate. Cureus. 2020;12(5):e8092.

Tisack A, Fotouhi A, Fidai C, Friedman BJ, Ozog D, Veenstra J. A clinical and biological review of keratoacanthoma. Br J Dermatol. 2021;185(3):487-98.

US Environmental Protection Agency (US EPA), Office of Prevention, Pesticides and Toxic

Substances. Blondell J, Hawkins MS. Review of piperonyl butoxide incident reports. DP Barcode D302303, Chemical #067501. EPA Series 361, 2004.

US Environmental Protection Agency (US EPA), Cancer Assessment Review Committee, Health Effects Division Office of Pesticide Programs. Evaluation of the carcinogenicity potential of Pyrethrins (fourth review), PC Code 069001, 2008.

Wagner SL. Fatal asthma in a child after use of an animal shampoo containing pyrethrin. West J Med. 2000 Aug;173(2):86-7.

Wax PM, Hoffman RS. Fatality associated with inhalation of a pyrethrin shampoo. J Toxicol Clin Toxicol. 1994;32(4):457-60.

Zehringer, M., Herrmann, A. Analysis of polychlorinated biphenyls, pyrethroid insecticides and fragrances in human milk using a laminar cup liner in the GC injector. Eur Food Res Technol. 2001;212:247–51.

Zito PM, Scharf R. Keratoacanthoma. [Updated 2022. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499931/

APPENDIX VII: STUDY SUMMARIES

1) CAR studies summary

a) Summary studies for toxicology used for classification:

Doc IIIA Sections 1-6 PYRETHRINS PT18 (BRA, MGK and SCJ)

Doc III-A Sections 1-6 Chrysanthemum PT18 (KPIC)

b) Summary studies for degradation and ecotoxicology used for classification:

Doc IIIA Sections 7-9 PYRETHRINS PT 18_BRA

Doc IIIA Sections 7-9 PYRETHRINS PT 18_KPIC

IIIA7.1.2.1.1 Hein_Moendel 2017

IIIA7.2.1 Fifi 2015 a, b and c

IIIA7.2.1 Hein 2017

IIIA7.2-Perboni

IIIA7.4.1.2 - 5 Pyrethrin metabolites_Daphnia_Mantilaci

IIIA7.4.1.2 - Pyrethrum Extract_6 Pyrethrin esters_Daphnia_Mantilacci

IIIA7.4.3.5.1 - Pyrethrum Extract Pale 50%_Chironomus

IIIA7.4.3.5.1 - Pyrethrum Extract Pale 50%_Chironomus

IIIA7.4.3.5.1 - Pyrethrum Extract Pale 50%_Chironomus

2) Studies included in the report submitted for the EU peer review of active substances used in plant protection products (RAR)

a) All these studies have been already evaluated under BPR in the present CAR and corresponding docs IIIA above mentioned, hence there is no further evaluation under RAR presented in this appendix, except for the following studies, whose summaries are included in document "Pyrethrins_RAR_Ecotoxicology studies summary not in CAR", attached below:

ES *Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

Test organism	**Test substace**	**Duration, conditions**	**Toxicological endpoint**	**Title**	**Dossier file number**
Rat; Sprague-Dawley	^{14}C-Pyrethrin 1; 97.81%	Single oral dose by gavage; 3 days observation period	Multiple applied high doses of ^{14}C-pyrethrin 1 decrease the excretion in urine and possibly its metabolism rate to chrysanthemum dicarboxylic acid.	Determination of the metabolic profile of ^{14}C-Pyrethrin 1 in rats when administered in four different dosing formulations. GLP, Unpublished	CA 5.1.1/03 Limoges, J. 1994
Mouse or rat liver microsomes	Pirethrin I & Pirethrin II	1 h incubation	No metabolites were found of toxicological concern.	Pyrethroid metabolism; Microsomal oxidase metabolites of (s)-bioallethrin and the six natural pyrethrins. Not GLP, Unpublished	CA 5.1.2/01 Class, T.J., Ando, T., & Casida, J.E. 1989
Rat, dog and human hepatocytes	[cyclopentenone-2-^{14}C]Pyrethrin 1; 97.9%	0, 30, 60, 120, 180 or 240 min. incubation	There is no strong evidence for any radiolabelled unique or disproportionate human metabolites.	[cyclopentenone-2-^{14}C]Pyrethrin 1: Comparative *In Vitro* Metabolism Using Rat, Dog and Human Hepatocytes GLP, Unpublished	CA 5.1.2/02 Paul, D. 2020
Rat; Sprague-Dawley	Pyrethrum Extract; 55.99%	Acute oral gavage; 14 days observation period	LD50 : Males : 3.81 g/kg bw. Females : 1.21 g/kg bw.	Acute oral toxicity, LD50-rats (Pyrethrum Extract 55.99%) Not GLP, Unpublished	IIA 5.2.1/03 Costello, B.A. 1986
Rabbit; New Zealand White	Pyrethrum Extract	6 h/day; 5 days consecutively	Slight to moderate erythema, whereas the formation of edema generally does not appear.	Letter: An exploratory 5-day dermal irritation study in New Zealand white rabbits using pyrethrum extract. Not GLP, Unpublished	IIA 5.2.4/02 Schoenig, G.P. 1991
Rat; COBS® CD®	Pyrethrum Extract; 57.57%	10 days consecutively; once/day	Dose levels of 5, 25 and 75 mg/kg bw/day are considered suitable for use in the definitive teratology study	Pyrethrum Extract, Range-Finding Teratology Study in Rats. GLP, Unpublished	CA 5.6.2/01 Schardein, J.L. 1987a
Rabbit; New Zealand White SPF	Pyrethrum Extract; 57.57%	13 days consecutively; once/day	Dose levels of 25, 100 and 250 mg/kg bw/day are considered suitable for use in the definitive teratology study.	Pyrethrum extract Range-Finding Teratology Study in Rabbits. GLP, Unpublished	CA 5.6.2/02 Schardein, J.L. 1987b
Rat; Charles River CD® (Sprague-Dawley)	Pyrethrum Extract; 57.47%	Acute oral gavage; 24 h observation period	A dosing solution containing 10% total pyrethins and dose levels of 0.04, 0.125, and 0.4 g total pyrethrins/kg bw were selected for males. For females in the definitive acute neurotoxicity study, a dosing solution containing 5% total pyrethins and dose levels of 0.02, 0.063,	Peroral (Gavage) Neurotoxicity Probe Study with Pyrethrum Extract in CD® Rats. GLP, Unpublished	CA 5.7.1/01 Hermansky, S. J. & Hurley, J. M. 1993a

			and 0.2 g total pyrethrins/kg bw were selected. In addition, 3 to 5 h was determined as the time for the first post-exposure evaluation time.		
Rat; Charles River Crl:CD® (SD)IGS BR	Pyrethrins	Acute oral gavage, 24 h observation period; observed twice daily	Pyrethrins are considered to be the least potent type I pyrethroid (Type 1-T syndrome – non-α-cyano substituent in the 3-phenoxybenzyl alcohol moiety of the molecule) based on FOB data. Pyrethrins are of significantly lower neurotoxicity than all other pyrethroid molecules tested.	Comparative functional observational battery study of twelve commercial pyrethroid insecticides in male rats following acute oral exposure. GLP, Unpublished	CA 5.7.1/03 Weiner, M.L., Nemec, M., Sheets, L., Sargent, D. & Beckenridge, C. 2009

b) All these studies have been already evaluated under BPR in the present CAR and corresponding docs IIIA above mentioned, hence there is no further evaluation under DAR presented in this appendix, except for the following studies, whose summaries are included in document "Pyrethrins_DAR_Ecotoxicology studies summary not in CAR", attached below:

Test organism	**Test substace**	**Duration, conditions**	**Toxicological endpoint**	**Title**	**Dossier file number**
Fish, acute and chronic					
Danio rerio	Pyrethrum extract (FEK-99)	96h flow through	LC_{50} = 19.8 µg /L	Fish acute toxicity test over 96 h under flow through conditions (OECD TG 203, 1992) Acute toxicity of refined pyrethrum concentrate on the zebrafish (Danio rerio) GLP	KCA 8.2.1/04 Teigeler 2013b GAB-034/4-32/A
Gasterosteus aculeatus	Pyrethrum extract (FEK-99)	96h flow through	LC_{50} = 10.9 µg /L	Fish acute toxicity test over 96 h under flow through conditions (OECD TG 203, 1992) Acute toxicity of refined pyrethrum concentrate on the three-spined stickleback (Gasterosteus aculeatus)	KCA 8.2.1/05 Teigeler 2013a GAB-034/4-32/G
Cyprinodon variegatus	Pyrethrins TGAI	33 days, flow through	NOEC = 3.5 µg a.s./L	Pyrethrins TGAI - Early Life-Stage Toxicity Test with Sheepshead Minnow (Cyprinodon variegatus) Following OPPTS Draft guideline 850.1400	KCA 8.2.2.1/02 Lee, M.R. 2012
Invertebrates, acute and chronic					
Mysidopsis bahia	Pyrethrum extract (FEK-99)	96h flow through	LC_{50} = 1.4 µg /L (measured concentration)	Pyrethrum extract (FEK-99) – acute toxicity to mysid shrimp (Mysidopsis bahia) under flow-through conditions GLP, Unpublished	IIA, 8.3.1.3/01 (B) Machado M.W 1994
Crassostrea virginica	Pyrethrum extract (FEK-99)	96h flow through	EC_{50} = 87 µg /L (measured concentration)	Pyrethrum extract (FEK-99) – acute toxicity to eastern oyster (Crassostrea virginica) under flow-through conditions GLP, Unpublished	IIA, 8.3.1.4/01 (B) Dionne E 1994

Americamysis bahia	Pyrethrum Stewardship Blend	28 days, flow through	NOEC = 0.25 µg pyrethrins/L	Pyrethrum Stewardship Blend – Life Cycle Toxicity Test with Mysids (Americamysis bahia) Following Draft OPPTS Guideline 850.1350	KCA 8.2.5.2/01 Lee, M.R. 2013
Algae					
Selenastrum capricornutum	Pyrethrum extract	72h static	E_bL_{50} >56,7 mg/L (measured concentration) E_rL_{50} > 1,95 mg/L (measured concentration)	Refined pyrethrum extract – Algal growth inhibition assay GLP, Published	IIA, 8.4/01 (B) Jenkins C.A 2003
Scenedesmus subspicatus	Pyrethrum extract	72h static	EC_{50} > 2,32 mga.s/L (nominal concentration) EC_{50} > 1,27 mga.s/L (measured concentration)	Natural Pyrethrum: algal inhibition test GLP, Published	IIA, 8.4/02 (B) Mead C, McKenzie, J. 2003
Water/sediment organisms					
Hyalella azteca	Pyrethrum Stewardship Blend	96h flow through	LC50 = 0.76 µg/L	Pyrethrum Stewardship Blend - Acute Toxicity to Freshwater Amphipods (Hyalella azteca) Under Flow-Through Conditions	KCA 8.2.8/01 Bradley, M.W., 2013

DAR - ANNEX B.6 Toxicology and metbolism

Active substance: Pyrethrins

1) Determination of the metabolic profile of 14C-Pyrethrin 1 in rats when administered in four different dosing formulations; Limoges J., 1994; Cross reference IIA 5.1.1/03

Guidelines:

US EPA 85-1

GLP:

Yes, conducted under GLP/Officially recognised testing facilities.

The study is acceptable as additional data

Executive Summary:

Absorption and excretion patterns and the metabolic profile of Pyrethrin 1 were investigated when administered orally to male rats in the form of four different dosing formulations. Eight male rats were distributed into four groups. Three groups were administered 100 mg ^{14}C-pyrethrin 1/kg bw in corn oil, or DMSO (5 mL of dosing mixture/kg bw) by gavage. In the fourth group rats were administered orally 4 doses of 400 mg ^{14}C-pyrethrin 1/kg bw in DMSO at 12-hour intervals.

The mean percent of administered radioactivity found in the urine from the rats in the various dosing regimens in which ^{14}C-Pyrethrin was administered in corn oil or DMSO ranged from 22.80% to 33.80%. The corresponding value for the group of animals administered ^{14}C-Pyrethrin 1 as a food slurry was 27.69%. The mean percent of administered radioactivity found in the faeces from the rats in the various dosing regimens which did not involve administration of ^{14}C-Pyrethrin 1 as a food slurry ranged from 55.01% to 63.20%. The corresponding value for the rats administered ^{14}C-Pyrethrin 1 as a slurry in food was 38.37%. Except for the group of rats administered ^{14}C-Pyrethrin 1 as a slurry in food (group 2), total recovery of radioactivity ranged from 84.28% to 90.44%. Total recovery in group 2 was 66.06%.

HPLC analysis of the urine revealed that all groups, regardless of the dosing regimen or vehicle used to administer the dose, produced the same major metabolites. However, when comparing the percent of the total radioactivity represented by chrysanthemum dicarboxylic acid (CDCA) in the urine from each group, the values are 47.52, 42.17, 41.26, and 26.77% for the single dose corn oil, single dose food slurry, single dose DMSO and multiple dose DMSO groups, respectively.

The vehicle (corn oil, food slurry, or DMSO) had little or no influence on the percent dose excreted in the urine or faeces or the percent dose represented by CDCA in the urine. The results indicate that multiple applied high doses of ^{14}C-Pyrethrin 1 decrease the excretion rate in urine and possibly its metabolism rate to chrysanthemum dicarboxylic acid as well.

Materials and methods:

A. MATERIALS

1. Test Material 1: ^{14}C-Pyrethrin 1

Purity: 97.81%

Specific activity: 54 mCi/mmol

Specification code: CFQ.7422

Test Material 2: Non-radiolabelled pyrethrin 1

Purity: 98.9%

Specification code: NK9304

Vehicle and/or positive: Mazola corn oil, DMSO, and a food slurry

2. Test Animals

Species: Rat

Strain: Sprague-Dawley (Crl:CD VAF)

Age: 7 weeks

Sex: Males

Weight at dosing: 275-370 g

Source: Charles River Breeding Laboratories, Portage, Michigan.

Acclimation period: A minimum of 7 days

Diet: Purina Certified Rodent Chow #5002 ad libitum

Water: Tap water ad libitum

Housing: Individually housed in stainless steel suspended cages

Environmental Conditions

Temperature: 62°F -71°F

Humidity: 13-62%

Air changes: A minimum of 7 per hour

Photoperiod: 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

7. Dates of work: 05 November 1993 to 13 December 1994

8. Animal assignment and treatment

Eight male rats (2/group) were administered a single oral dose of 100 mg ^{14}C-Pyrethrin 1/kg bw in Mazola corn oil, food slurry, or DMSO (5 mL of dosing mixture/kg bw) by gavage. In one group rats were administered 4 doses of 400 mg 14C-Pyrethrin 1 each/kg bw in DMSO at 12-hour intervals.

Group no.	Males/group	^{14}C-Pyrethrin 1 dosage level	Dose	Route of

		mg/kg bw	**formulation**	**administration**
1	2	100	Corn oil	Oral, single
2	2	100	Food slurry	Oral, single
3	2	100	DMSO	Oral, single
4	2	4 x 400	DMSO	Oral, multiple

9. Administration of the dosing solution

The dosing solutions were delivered by gavage with an intubation needle equipped with a disposable syringe. All doses for groups 1, 2, 3, and 4 were administered on a constant volume basis, i.e. 5 mL of dosing mixture per kg of body weight. The actual amount of compound administered to each rat was measured as a differential between the weight of the syringe before and after dosing.

10. Observations and sampling

Urine, faeces, and water rinses were collected at the following time intervals: 0-24, 24-48, and 48-72 hours after administration and analysed for total radioactivity to determine excretion patterns. The urine was analysed for Pyrethrin 1 and chrysanthemum dicarboxylic acid. Sample combustion was performed by a Harvey Sample Oxidizer, LSC by a Beckmann LS spectrometer and metabolite characterisation in urine by HPLC.

11. Statistics

The mean and standard deviation were used to characterize the data where appropriate.

Results and discussion:

1. Observations

All rats survived, and no signs of toxicity were observed in groups 1 to 3 (single doses of 100 mg/kg ^{14}C-Pyrethrin 1). In the multiple dose group (4 x 400 mg/kg ^{14}C-Pyrethrin 1) animals reacted with twitching (during the first 5 hours after the first treatment) and spasms (during the first two hours after the first treatment). Afterwards no clinical signs of toxicity were noted, also not after redosing at hours 12, 24, and 36.

Table 6.1.1/03-1: Mean body weights, compounds and radioactivity of males and females in a preliminary blood kinetics test

Group no.	**^{14}C-Pyrethrin dosage level mg/kg bw**	**Dose formulation**	**Mean body weight (kg) ± SD**	**Mean administered dose ±SD**	
				mg Pyrethrin/kg	Total µCi
1	100	Corn oil	0.302±0.004	94.28±0.13	14.6±0.1
2	100	Food slurry	0.345±0.011	93.38±1.97	12.9±0.1
3	100	DMSO	0.296±0.029	100.59±0.28	14.2±1.3
4	4 x 400[1]	DMSO	0.350±0.029	1547.08±22.46	28.6±2.0

[1]Total of four doses, administered at 12-hour intervals.

2. Excretion

The total recovery of radioactivity excreted in urine and faeces was generally ranged from 84 to 90%, however, in group 2 (100 mg/kg in food slurry) the recovery was less due to

incomplete transfer of dose from the syringe to the animals caused by the nature of the food slurry. A higher proportion of radioactivity was excreted in the faeces (38 to 63% of applied radioactivity) than in urine (23 to 34% of applied radioactivity). After single dose applications the excretion rate of radioactivity was highest during the first 48 hours.

Table 6.1.1/03-2: A comparison of total recovery of radioactivity expressed as a cumulative percent of administered radioactivity excreted in urine and faeces from rats administered various dose formulations

Sample	Group 1 (Mean ± SD)	Group 2 (Mean ± SD)	Group 3 (Mean ± SD)	Group 4 (Mean ± SD)
Urine	29.28 ± 1.99	27.69 ± 2.67	33.80 ± 1.51	22.80 ± 0.25
Faeces	55.01 ± 0.50	38.37 ± 5.81	56.64 ± 1.78	63.20 ± 13.72
Total recovery	84.28 ± 2.49	66.06 ± 3.14	90.44 ± 3.29	86.00 ± 13.97

Analysis of urine using HPLC from groups 1, 2, and 3 showed that 1.8 to 13.47% of administered radioactivity were excreted as chrysanthemum dicarboxylic acid (CDCA). However, in the urine from group 4, CDCA represented 4.74% of administered radioactivity.

Table 6.1.1/03-3: Amount of CDCA in the urine from male rats in various dose formulations

Group no.	% of dosed radioactivity present in the urine sample	% of radioactivity in urine as CDCA	% of dosed radioactivity excreted in urine as CDCA
1	28.35	47.52	13.47
2	26.50	42.17	11.18
3	29.81	41.26	12.30
4	17.69	26.77	4.74

Conclusion:

It is concluded that the vehicle (corn oil, food slurry, or DMSO) has little or no influence on the percentage of dose excreted in the urine of faeces or the percentage of dose represented by CDCA in the urine. On the other hand, the results indicate that multiple applied high-doses of ^{14}C-Pyrethrin 1 decrease the excretion in urine and possibly its metabolism rate to chrysanthemum dicarboxylic acid as well.

A. ASSESSMENT AND CONCLUSION BY APPLICANT

Assessment:

This was a GLP compliant study. The study report is in broad compliance with OECD 417, although the guidelines are not mentioned in the study report.

Conclusion:

The vehicle had little or no influence on the percentage dose excreted in the urine or faeces or the percentage of dose represented by CDCA in the urine. The results indicate that a lower percentage of the total applied radioactivity was excreted in urine following multiple high doses of ^{14}C-pyrethrin 1, and the proportion of radioactivity excreted in urine as chrysanthemum dicarboxylic acid was also lower, when compared to percentages of dosed radioactivity excreted in urine and as CDCA, following a single low dose.

B. ASSESSMENT AND CONCLUSION BY RMS

The study was evaluated in the original DAR (2007) and was considered acceptable as additional data.

It is concluded that the vehicle has little or no influence on the percentage of dose excreted in the urine or feces or the percentage of dose represented by CDCA in the urine. On the other hand, the results indicate that multiple applied high doses of ^{14}C-pyrethrin 1 decrease the excretion in urine and possibly its metabolism rate to chrysanthemum dicarboxylic acid as well.

2) *Pyrethroid metabolism; Microsomal oxidase metabolites of (S)-bioallethrin and the six natural pyrethrins; Class T.J., Ando T., and Casida J.E., 1989; Cross reference IIA 5.1.2/01*

Guidelines:

Non-guideline study

GLP:

No, not conducted under GLP/Officially recognised testing facilities.

The study is acceptable

Executive Summary:

Six natural pyrethrins were isolated from purified extract and were analysed together with their synthetic analog (S)-bioallethrin to describe the production of possible metabolites. A complete metabolic profile was developed which shows similarities in the metabolization of natural chrysanthemates (cinerin 1, jasmolin 1, pyrethrin 1) and pyrethrates (cinerin 2, jasmolin 2, pyrethrin 2). The metabolism of the chrysanthemates proceed mainly through oxidative processes while the pyrethrates are metabolised through a combination of hydrolytic and oxidative processes. No metabolites of toxicological concern were found.

Materials and methods:

A. MATERIALS

1. Test Material 1: (S)-bioallethrin (synthetic analogue)

Test Material 2: Cinerin 1

Test Material 3: Jasmolin 1

Test Material 4: Pyrethrin 1

Test Material 5: Cinerin 2

Test Material 6: Jasmolin 2

Test Material 7: Pyrethrin 2

2. Mammalian metabolic system

System: Mouse or rat liver microsomes

3. Test animals

Species: Rat

Strain: Albino

Sex: Male

Weight at dosing: Not stated

Source: Not stated

Acclimation period: Not stated

Diet: Not stated

Water: Not stated

Housing: Not stated

Environmental Conditions

Temperature: Not stated

Humidity: Not stated

Air changes: Not stated

Photoperiod: Not stated

B. STUDY DESIGN AND METHODS

1. Dates of work: 19 November 1992 to 19 December 1995

2. Method

The pyrethrum extract constituents and (S)-bioallethrin were analysed by high resolution gas chromatography (HRGC) with an electron capture detector (ECD) of HRGC-chemical ionization (CI)-mass spectrometry (MS). These methods are applied here to the rethrin metabolites after appropriate derivations. This study compares the metabolic fate of (S)-bioallethrin and the six natural pyrethrins in mouse and rat liver microsomal oxidase systems and of (S)-bioallethrin in rats.

a) Formation and analysis of microsomal metabolites

The substrate (0.1 µmol) was incubated with mouse or rat liver microsomes (0.1, 0.3, 1.0, 3.0 mg protein) and NADPH (0 or 2.4 µmol) in phosphate buffer (0.1 M, pH 7.4, 2 mL) for 1 hour at 37 ºC. Following extraction of the aqueous and organic phases, analysis was conducted using HRGC coupled to CI–MS. The amount of substrate recovered was determined by HRGC-ECD relative to the internal standard. Recoveries of the chrysanthemates were 100 ± 10% at 0.3 mg microsomal protein in the absence of NADPH. The extent of metabolism of the chrysanthemates was calculated from the loss of substrate with NADPH.

The microsomal metabolism of the pyrethrates in the presence and absence of PSCP (phenyl saligenin cyclic phosphonate, a potent esterase inhibitor) was also investigated. The extent of metabolism was determined by substrate loss for incubations with microsomes compared with no microsomes. Another investigation compared the extent of metabolism of the (E)-8' vs. (Z)-8' isomers of cinerin 1, jasmolin 1 and pyrethrin 1 incubated as a mixture with bioallethrin each at 0.014 µmol per incubation with 0.3 and 1.0 mg mouse microsomal protein and NADPH.

b) Formation and analysis of urinary metabolites

Male albino rats (not specified) were treated with (S)-bioallethrin orally by stomach tube (250 mg/kg followed after 6 hours with 500 mg/kg) or intraperitoneally (12.5 mg/kg followed after 6 hours with 25 mg/kg) using DMSO as the vehicle. No signs of toxicity were observed. Urine was collected 0-6 hours after the second treatments were analysed for free and conjugated urinary metabolites.

3. Abbreviations for chemicals

The abbreviations ol, al and acid. refer to alcohols, aldehydes and carboxylic acids, respectively. Trimethylsilyl ethers are designated as TMS, ethyl esters as Et, epoxides as epoxy, diols from hydrolysis of epoxides as dihydrodiol, and their TMS derivatives as dihydro (TMS)2. Designations such as 5/6-ol and 6'/10'/11'-ol indicates that the available information does not differentiate among the specified positions. "PI" and "PII" refer to rearranged products shown in Figure 6.1.2/01-1 and 5.1.2/01-2.

Results and discussion:

1. Microsomal metabolites

Metabolism of the chrysanthemates (S)-bioallethrin, cinerin 1, jasmolin 1, and pyrethrin 1 by NADPHdependent oxidases of mouse liver microsomes yields 13-18 metabolites in each case oxidized at the methyl, methylene, and alkenyl substituents to form alcohols, aldehydes, carboxylic acids, epoxides and dihydrodiols. Rat microsomes are more specific than rat mouse microsomes in hydroxylating the (E)-methyl substituent of the 2-methylpropenyl moiety compared with other molecular sites.

Table 6.1.2/01-1: Extent of oxidative metabolism by mouse and rat liver microsomes for (S)-bioallethrin (A), the six natural pyrethrins and the (E)-8'-isomers of C1, J1 and P1

Designation	NADPH-dependent loss of substrate, %[a], at indicated level of microsomal protein (mg)						
	Mouse				Rat		
	0.1	0.3	1.0	3.0	0.3	1.0	3.0
A	25	50[b]	80	95	20	50	60

C1c(C2)d	15	45	90 (93)[d]	-	-	75	-
J1c(J2)d	10	35	80 (98)[d]	90	30	50	60
P1c(P2)d	10	40	80 (99)[d]	-	-	50	-

A (S)-bioallethrin

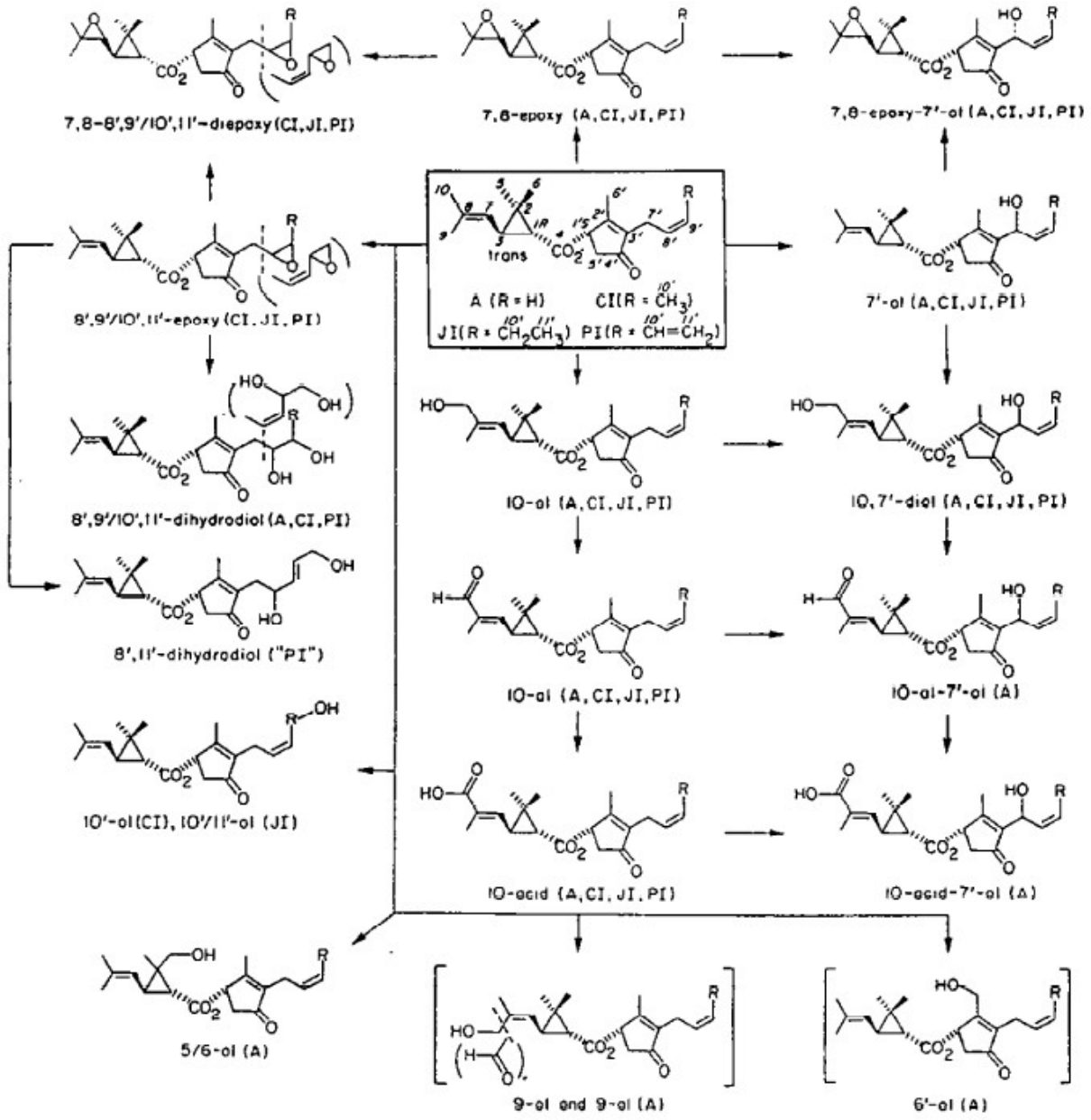
[a] Mean of two experiments differing by <10%

[b] Substrate loss (%) with primary metabolites used as substrates: A-7.8-epoxy [7S)-isomer] 35, [(7R)-isomer] 10; A-7'-ol 35 with no diastereomer difference; A-10-ol 25; A-10-al 30.

[c] Mean o experiments of pyrethrates with NADPH±PSCP, metabolism of C2, J2 and P2 in the absence of NADPH is 25, 55 and 55% respectively, with PSCP and 55, 65 and 75%, resepectively, without PSCP.

C=cinerin, J=jasmolin, P=pyrethrin

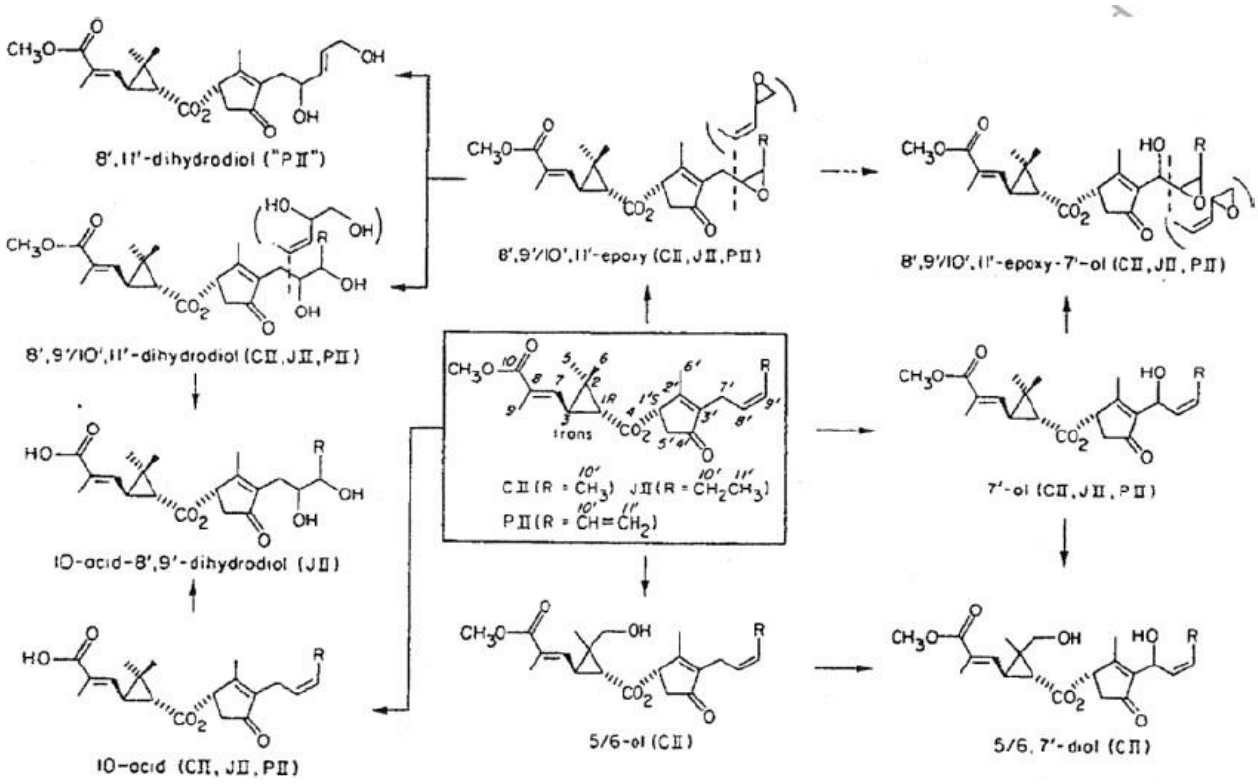
Figure 6.1.2/01-1: Partial metabolic pathways for (S)-bioallethrin (A), cinerin 1 (C1), jasmolin 1 (J1), pyrethrin 1 (P1) in mouse and rat liver microsomal oxidase systems an of (S)-bioallethrin in rats *in vivo*. Additional metabolites not designated as structures arise from other combinations of modifications in the acid and alcohol moieties. Brackets designate compounds tentatively identified from SeO_2-oxidation and indicated but not established as metabolites



2. Urinary metabolites

Metabolites in the urine of allethrin-treated rats include compounds modified in both the 2-methylpropenyl and allyl moieties as free carboxylic acids and glucuronides. The pyrethrates cinerin 2, jasmolin 2, and pyrethrin 2 undergo microsomal hydrolysis of the methoxycarbonyl group and oxidation of the butenyl, pentenyl, and pentadienyl substituents to alcohols, epoxides, and dihydrodiols.

Figure 6.1.2/01-2: Partial metabolic pathways for cinerin 2 (CII), jasmolin 2I (JII) and pyrethrin 2I (PII) in mouse liver microsomal oxidase systems. Although not observed, 10', 11'-epoxy-PI is included as a likely intermediate



Conclusion:

A complete metabolic profile was developed which shows similarities in the metabolism of natural chrysanthemates and of pyrethrates. The metabolism of the chrysanthemates proceeds mainly through oxidative processes while the pyrethrates are metabolised through a combination of hydrolytic and oxidative processes. No metabolites were found of toxicological concern.

A. ASSESSMENT AND CONCLUSION BY APPLICANT

Assessment:

The study report is in broad compliance with OECD 417, although the guidelines are not mentioned in the study report. The study provides reasonable information to predict the absorption, distribution, and excretion of the natural Pyrethrins.

Conclusion:

Agreement with the conclusions of the study authors.

B. ASSESSMENT AND CONCLUSION BY RMS

The study was evaluated in the original DAR (2007) and was considered acceptable.

A complete metabolic profile was developed which shows similarities in the metabolism of

natural chrysanthemates and of pyrethrates. The metabolism of the chrysanthemates proceeds mainly through oxidative processes while the pyrethrates are metabolised through a combination of hydrolytic and oxidative processes. No metabolites were found of toxicological concern.

3) [cyclopentenone-2-^{14}C]Pyrethrin 1: Comparative In Vitro *Metabolism Using Rat, Dog and Human Hepatocytes; Paul D., 2020; Cross reference IIA 5.1.2/02*

Guidelines:

No detailed test guidelines for the conduct of comparative *in vitro* metabolism studies are currently available. The data requirement is based on the Commission Regulation (EU) No 283/2013, 5.1.1, in accordance with Regulation (EC) No 1107/2009.

GLP:

Yes, UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994); OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17; EC Commission Directive 2004/10/EC

The study is acceptable

Executive Summary:

The purpose of this study is to compare the metabolism of [cyclopentenone-2-^{14}C]Pyrethrin 1 also written as [^{14}C]Pyrethrin 1 using rat, dog and human hepatocytes.

[^{14}C]Pyrethrin 1 (10 µM) was incubated (at 37 ± 1 ºC) with either rat, dog or human hepatocytes (0.5 x 106 viable cells/mL for all species) for 0, 30, 60, 120, 180 or 240 minutes. Incubations in the absence of hepatocytes were also conducted with [^{14}C]Pyrethrin 1 for 0 and 240 minutes, to check the stability under incubation conditions. Incubation samples were analysed by HPLC with on-line radioactive monitoring. The proportions of metabolites produced and parent [^{14}C]Pyrethrin 1 were quantified.

A summary of the metabolites detected is presented in Summary Table 6.1.2/02-1.

Table 6.1.2/02-1 Metabolites detected following incubation of Pyrethrin 1 (10 µM) with Rat, Dog and Human Hepatocytes after 30 to 240 minutes

Metabolite fraction designation	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R10**	**R11**	**R12**	**R13**	**R14**
Rat	+	++	+	-	++	+	++	+	+	++	+	-	+	+
Dog	+	+	-	-	++	+	++	+	+	++	+	++	+	+
Human	-	-	-	-	+	+	++	-	+	++	+	+	+	+

Metabolite fraction designation	**R15**	**R16**	**R17**	**R18**	**R19**	**R20**	**R21**	**R22**	**R23**	**R24**	**R25**	**R26**	**R27**	**[^{14}C]Pyrethrin 1 metabolism (%)†**
Rat	+	-	-	-	+	+	++	+	-	+	+	-	-	> 99
Dog	-	+	++	+	++	-	++	-	+	+	+	+	+	96.1
Human	-	-	+	-	++	-	++	+	+	-	+	+	-	84.6

+ Present at between ≥ 1% and < 5% sample radioactivity in species
++ Present at ≥ 5% sample radioactivity in species
- Not detected at < 1% or above sample radioactivity in species
† Consumption of parent at 240 minutes compared to time zero
* present in rat and dog at earlier timepoints.

Materials and test methods:

A. MATERIALS

1. Test substance [cyclopentenone-2-^{14}C]Pyrethrin 1

2. Chemical name (IUPAC) [(1S)-2-methyl-4-oxo-3-[(2Z)-penta-2,4-dienyl]cyclopent-2-en-1-yl]

(1R,3R)-2,2-dimethyl-3-(2-methylprop-1- enyl)cyclopropane-1-carboxylate

3. CAS number 121-21-1

4. Molecular formula $C_{21}H_{28}O_3$

5. Molecular weight 328.45

6. Lot number LY13TX/NP/01

7. Specific activity 2.11 GBq/mmol

8. Radioactive concentration 0.345 MBq/mL

9. Radiochemical purity 97.9% (HPLC)

10. Physical form Solution in Acetonitrile

11. Storage conditions -10 °C to -30 °C, under nitrogen and protected from light

B. STUDY DESIGN AND METHODS

All cryopreserved hepatocytes were obtained from BioIVT (formerly Celsis IVT and Bioreclamation IVT) and delivered stored frozen in liquid nitrogen. Details of the hepatocytes used in this study are as follows:

Species	Strain	Gender	Batch number	Number of donors	Number of vials used
Rat	Sprague Dawley	Male	VRO	9	2
Dog	Beagle	Male	SDS	3	3
Human	Not applicable	Mixed	QGS	10	2

All animal hepatocytes were supplied as a pool of male donors. Each vial used contained at least 5 million cells. Human hepatocytes were supplied as a mixed-gender pool.

1. Incubation of [^{14}C]Pyrethrin 1 with Rat, Dog, and Human Cryopreserved Hepatocytes

Incubations of [^{14}C]Pyrethrin 1 were conducted with rat, dog and human cryopreserved hepatocytes as follows:

- Concentration: 10 µM
- Incubation times: 0, 30, 60, 120, 180 and 240 mins
- Incubation temperature: 37 ± 1 ºC
- Number of replicates: Two
- Cell concentration: 0.5 x 10^6 viable cells/mL incubation medium
- Volume of incubation medium: Either 5.0 mL (hepatocyte-containing samples or 2.0 mL (no hepatocytes samples)

The incubation components were mixed together in well-plates (6-well format) for each sample as follows:

- Supplemented Williams' Medium E containing 2.5 × 106 viable cells in 4950 µL
- [^{14}C]Pyrethrin 1 (50 µL of 1 mM solution in acetonitrile) for hepatocyte-containing samples.

Following the final addition of [^{14}C]Pyrethrin 1, 0 minutes samples were terminated immediately. The well-plates were then placed on a tilting mini rocker-shaker in a temperature-controlled incubator (set at 37 ºC) to commence the incubation.

The following control incubations were also conducted in parallel:

- Incubation of [^{14}C]Pyrethrin 1 for 0 or 240 minutes in the absence of hepatocytes (duplicate incubations at 10 µM),
- Positive control samples incubating 7-ethoxy[3-^{14}C]coumarin ([^{14}C]7EC) at a concentration of 25 µM in single replicate for 0, 30, 60, 120, 180 and duplicate for 240 minutes.

At the end of requisite incubation period, an aliquot (0.5 mL) was removed from each incubate and transferred to an aliquot (0.5 mL) of chilled acetonitrile to stop the reaction. Each sample was stored on Ice and then treated using an ultrasonic bath for approximately 10 minutes to fully disrupt the cells. The samples were then processed as described in section

2. Analysis of [^{14}C]Pyrethrin 1 and Metabolites

i. Preparation of Reference Standards

Parent non-radiolabelled Pyrethrin 1 which was previously supplied at a concentration of 0.1 mg/mL in cyclohexane was dried down and then reconstituted in acetonitrile (0.5 mL) to remove the storage solvent. Reference standard, non-radiolabelled pyrethrolone was prepared by weighing 4 µL (equivalent to 4.12 mg) and dissolving in 1 mL of DMSO to give a pyrethrolone concentration of 4 mg/mL. The pyrethrolone solution was then diluted by combining an aliquot (100 µL) of the solution with 900 µL of purified water to obtain a concentration of 0.4 mg/mL.

ii. Processing of [^{14}C]Pyrethrin 1 Incubation Samples

Prior to analysis, 250 µL of each terminated incubation sample was centrifuged at 14,000 rpm (approximately 18,600 x g, 15 minutes, 5 ºC □ 3°C) to sediment the cell debris. Duplicate 10 µL aliquots were removed from the supernatant for LSC and 5 mL Ultima Gold added. The resulting supernatant (200 µL) was diluted with Pyrethrin 1 standard (0.1 mg/mL) 2.5 µL, purified water (600 µL) and metabolite reference standard pyrethrolone (0.4 mg/mL) 2.5 µL in a glass HPLC vial. The mixture was vortexed thoroughly, prior to transfer duplicate 25 µL aliquots were removed for LSC with 5 mL Ultima Gold added. The HPLC vial was transferred to an HPLC autosampler for injection (500 µL) into the HPLC. Samples were stored at approximately -70 ± 10°C prior to and on completion of analysis.

3. Characterisation of Isolated Hepatocytes

i. Determination of Initial Cell Viability by the Trypan Blue Exclusion Test

A solution of 0.08% (w/v) trypan blue was prepared by diluting 0.4% (w/v) trypan blue 1:4

(v/v) with supplemented Williams' Medium E. For each species, an aliquot (25 µL) of the initial hepatocyte cell suspension was mixed with an aliquot (50 µL) of 0.08% (w/v) trypan blue solution and an aliquot (175 µL) of supplemented Williams' Medium E. Each mixture was then loaded into a C-Chip haemocytometer (chamber depth 0.1 mm) and the number of viable and non-viable hepatocytes was determined in at least two 1 mm^2 grid areas. The total cell count for each 1 mm^2 area was multiplied by an appropriate scaling factor to give the total number of cells per mL and the mean value was calculated.

ii. Incubation of 7-Ethoxy[3-^{14}C]coumarin (7-EC) with Hepatocytes

In parallel incubations, positive control incubations were conducted with ([14C]7-EC) assubstrate. Incubations comprised of supplemented Williams' Medium, to give a final concentration of 7-EC of 25 µM in total volumes of either 5 mL or 4 mL. The final dimethylformamide (DMF) concentration was not greater than 1% (v/v). The incubations were performed in well-plates (6-well format) on a tilting mini rocker-shaker in a temperature-controlled incubator (set at 37 ºC). At the end of the requisite incubation period, an aliquot (1 mL) was removed from each incubate and transferred to an aliquot (1 mL) of chilled acetonitrile to stop the reaction. Each sample was stored on ice, then treated using an ultrasonic bath for approximately 10 minutes to fully disrupt the cells. All samples were then stored at *ca*. -20 ºC until sample processing. Following storage at *ca*. -20 ºC, the samples were transferred to clean labelled microcentrifuge tubes and centrifuged at 18,000 x g, at ca 4 ºC for 15 minutes to pellet the cell debris. The resulting supernatants from 7-EC samples were transferred to clean microcentrifuge tubes and concentrated to dryness under nitrogen gas. The dried residues were reconstituted in 40 mM ammonium formate (pH 5) and approximately half of each of the resulting supernatants was transferred to separate HPLC vials for direct injection into the HPLC system. A further portion of each solution (450 µL) was transferred to a clean micro-centrifuge tube along with a solution of β-glucuronidase enzyme (50 µL of a 40,000 units/mL solution, Type H1 from Helix pomatia also containing sulfatase activity). This mixture was incubated for 1 hour at 37 °C. Samples were then transferred to HPLC vials for direct injection into the HPLC system. Positive controls for β-glucuronidase and sulfatase enzyme activities were determined by the production of free phenolphthalein from phenolphthalein glucuronic acid and p-nitrocatechol from p-nitrocatechol sulfate, respectively, upon the addition of 1M sodium hydroxide after incubation (1 hour at 37 °C). Both the phenolphthalein glucuronic acid and p-nitrocatechol sulfate were prepared in 40 mM ammonium formate (pH 5).

Results and discussion:

For each chromatogram obtained from the analysis of samples from the incubations of [^{14}C]Pyrethrin 1 with rat, dog and human hepatocytes by HPLC, regions of radioactivity were assigned as metabolite fraction identities of R1 to R27 (each representing either a separated radioactive component or components where complete resolution could not be attained) and parent test item.

Not all metabolite fractions were present in every sample and some were only present in trace quantities. Regions of radioactivity that contained ≥ 5% of sample radioactivity were considered major (Whalley *et al*., 2017), whilst those that contained 1 to 4.9% of sample radioactivity were considered minor. Assigned regions of radioactivity that contained < 1% of sample radioactivity were considered below the limit of quantification.

1. Pyrethrin 1 Stability

The mean proportions of [^{14}C]Pyrethrin 1 (10 µM) remaining in the absence of hepatocytes were 97.3 and 86.7% following 0 and 240 minutes incubation, respectively. No notable

breakdown products were noted over this period.

2. Rat Hepatocytes

The mean proportions of [^{14}C]Pyrethrin 1 present in samples incubated with rat hepatocytes were 96.1, 28.8, 9.2 and 1.1% following 0, 30, 60 and 120 minutes incubation, respectively and BLQ thereafter. The mean extents of metabolism of [^{14}C] Pyrethrin 1 expressed relative to time zero were 67.3, 86.9, 95, > 99 and > 99% after 30, 60, 120, 180 and 240 minutes incubation, respectively. All metabolites R1 to R27 inclusive measured BLQ at time zero. In these samples up to 19 metabolites (R1-R3, R5-R11, R13-R15, R19-R22, R24 and R25) were detected above the limit of quantification at various time points during the incubation.

Metabolites R2, R5, R7, R10 and R21 were formed as major metabolites (≥ 5% of sample radioactivity) by rat hepatocytes and accounted for a mean sample radioactivity respectively of 8.5, 23.0, 10.5, 10.1 and 12.8% after 240 minutes incubation.

The other prominent metabolite fractions (minor metabolites) detected following incubation with rat hepatocytes, R1, R3, R6, R8, R9, R11, R13, R14 and R20 accounted for a maximum mean of 4.9% of the total sample radioactivity following 240 minutes incubation. The mean proportions of the metabolites detected above the limit of quantification generally increased with incubation time.

3. Dog Hepatocytes

The mean proportions of [^{14}C]Pyrethrin 1 present in samples incubated with dog hepatocytes were 97.9, 55.2, 31.1, 12.3, 5.0 and 1.8% following 0, 30, 60, 120, 180 and 240 minutes incubation, respectively. The mean extents of metabolism of [^{14}C]Pyrethrin 1 expressed relative to time zero were 42.7, 66.8, 85.6, 92.9 and 96.1% after 30, 60, 120, 180 and 240 minutes incubation, respectively. All metabolites R1 to R27 inclusive for both replicate samples measured at BLQ at time zero. In these samples up to 22 metabolites (R1, R2, R5-R14, R16-R19, R21, R23-R27) were detected above the limit of quantification at various time points during the incubation. Metabolites R5, R7, R10, R12, R17, R19 and R21 were formed as major metabolites (≥ 5% of sample radioactivity) by dog hepatocytes and accounted for a mean sample radioactivity respectively of 8.8, 8.4, 8.5, 5.3, 7.9, 15.8 and 5.4% after 240 minutes incubation. The mean proportions of the metabolites detected above the limit of quantification generally increased with incubation time.

4. Human Hepatocytes

The mean proportions of [^{14}C]Pyrethrin 1 present in samples incubated with human hepatocytes were 97.9, 77.3, 57.5, 28.4, 23.4 and 13.3% following 0, 30, 60, 120,180 and 240 minutes incubation, respectively. The mean extents of metabolism of [^{14}C]Pyrethrin 1 expressed relative to time zero were 20.6, 40.4, 69.5, 74.5 and 84.6% after 30, 60, 120, 180 and 240 minutes incubation, respectively. All metabolites R1 to R27 inclusive for both replicate samples measured at BLQ at time zero. In these samples up to 16 metabolites (R5-R7, R9-R14, R17, R19, R21-R23, R25 and R26) were detected above the limit of quantification at various time points during the incubation. Metabolites R7, R10, R19 and R21 were formed as major metabolites (≥ 5% of sample radioactivity) by human hepatocytes and accounted for a mean sample radioactivity respectively of 7.0, 5.7, 12.8 and 20.8% after 240 minutes incubation. The proportions of the metabolites detected above the limit of quantification generally increased with incubation time.

5. Comparison of Rat, Dog and Human Metabolism

Following the incubation of [^{14}C]Pyrethrin 1 with rat, dog and human hepatocytes over 240 minutes, the most prominent human metabolites were R7, R10, R19 and R21 (major). R10 seemed to corresponded to the reference standard Pyrethrolone chromatographically which is a major metabolite of [^{14}C]Pyrethrin 1. These metabolites were also observed in rat or dog hepatocytes. Metabolite R5 was a prominent major metabolite in the rat and dog, respectively, but a minor metabolite in the human. To a lesser degree, R8 was a minor metabolite in the rat and dog, respectively, and below the limit of quantification in human. Metabolite R2 was also a prominent major metabolite in the rat, a minor metabolite in the dog and below the limit of quantification in the human. Metabolite R19 was a prominent major metabolite in the dog and human, but present in the rat as a minor metabolite following 30 and 60 minutes incubation, respectively. R6, R9, R11, R13 and R14 were minor metabolites in all species. Taken together, there was no evidence for any radiolabelled unique human metabolites because metabolites identified following incubation with human hepatocytes were also detected in at least one animal species. In all species, the Phase I metabolite 7-hydroxycoumarin (7-HC) formed was subsequently conjugated to varying extents. The changes in the levels of the Phase I metabolite 7-HC following deconjugation indicated that all hepatocytes were metabolically viable and were capable of integrated Phase I/II metabolism under the incubation conditions used on this study. Therefore, the results generated for the incubation of these hepatocytes with [^{14}C]Pyrethrin 1 are considered to be valid.

Conclusion:

Extensive metabolism of [^{14}C]Pyrethrin 1-10 µM was observed across all species investigated with up to 27 metabolites (designated R1–R27) detected along with parent [^{14}C]Pyrethrin 1. [^{14}C]Pyrethrin 1 was metabolised to a slightly lesser extent in human hepatocytes than rat and dog hepatocytes in terms of the number of metabolites generated.

Following the incubation of [^{14}C]Pyrethrin 1 with rat, dog and human hepatocytes over 240 minutes, the most prominent metabolites in human hepatocytes were R7, R10 and R21 (major, >5%). Based on the UV chromatogram R10 corresponds to the reference standard Pyrethrolone which is a major metabolite of [^{14}C]Pyrethrin 1.

Taken together, there is no strong evidence for any radiolabelled unique or disproportionate human metabolites.

A. ASSESSMENT AND CONCLUSION BY APPLICANT

Assessment:

Unaudited interim report for the comparative in vitro metabolism study using rat, dog and human hepatocytes, with [cyclopentenone-2-^{14}C]Pyrethrin 1. The final report is to be issued in April 2020.

Conclusion:

Taken together, there is no strong evidence for any radiolabelled unique or disproportionate human metabolites.

B. Assessment and conclusion by RMS

The study was submitted for the renewal procedure and was considered acceptable.

There is no strong evidence for any radiolabelled unique or disproportionate human metabolites.

4) (A) Acute oral toxicity, LD50 – RATS (Pyrethrum Extract 55.99 %); Costello B.A., 1986;

Cross reference IIA 5.2.1/03

Guidelines:

Similar to 40 CFR, Sect. 163.81-1, Fed. Reg. , August 22, 1978; modified in accordance with revised EPA Pesticide Assessment Guideline:s, Nov. 1982.

GLP:

No

GLP was not compulsory at the time when the study was performed (1986)

The study is acceptable

Executive Summary:

A total of 50 (25 males and 25 females) albino rats, weighing 140-244 g, were administered a single dose of Pyrethrum Extract (55.99 % purity) by gavage. Following administration, the animals were allowed food and water ad libitum for the 14 day observation period. Animals were observed frequently on the day of dosing, twice per day on weekdays and once per day on weekends and holidays for signs of toxicity and mortality. Individual weights were recorded on the day of dosing, weekly thereafter and prior to sacrifice. After euthanasia, gross necropsies were performed on all animals.

Oral LD_{50}	males	= 3.81 g/kg bw
	femal	= 1.21 g/kg bw

Mortality started at dosages of 2510 mg/kg (females) and 1000 mg/kg (males). Females were more susceptible to Pyrethrum Extract than males. The main signs for intoxication were increased responsiveness to external stimuli, tremors, salivation and ruffled appearance. Gross pathology showed congested lungs (at males from 3980 mg/kg onwards, at females from 1260 mg/kg onwards).

Material and methods:

A. MATERIALS

1. Test Material: Pyrethrum Extract
   Description: Yellow liquid
   Lot/Batch #: FNB 86-2-18A
   Purity: 55.99 % Pyrethrins
   CAS #: As given in section 2
   Stability of test compound: Not determined
2. Vehicle and/or positive control: No vehicle
3. Test animals

Species:	Rat
Strain:	Outbred Sprague-Dawley
Age:	Young adult
Weight at dosing:	140-244 g
Source:	Buckshire Corp. Perkasie, PA 18944
Acclimation period:	At least 5 days
Diet:	Wayne Rodent-Blox 8604, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were housed in groups of 3-5 animals by sex in labeled stainless steel suspended cages.

Environmental conditions

Temperature:	21.1°C – 26.7°C
Humidity:	Relative humidity in %: 55 ± 25
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles, artificial fluorescent light

B. STUDY DESIGN AND METHODS:

1. In life dates: 09 June to 25 June 1986

2. Animal assignment and treatment

A total of 50 (25 males and 25 females) albino rats were administered a single dose of Pyrethrum Extract by gavage. Following administration, the animals were allowed food and water ad libitum for the 14 day observation period. Animals were observed frequently on the day of dosing, twice per day on weekdays and once per day on weekends and holidays for signs of toxicity and mortality. Individual weights were recorded on the day of dosing, weekly thereafter and prior to sacrifice. After euthanasia, gross necropsies were performed on all animals.

Table B.6. 14: Doses, mortality / animals treated

Dose (mg/kg bw)	Males	Females	Combined
630	-*	1/5	1/5
1000	0/5	1/5	1/10
1260	-	4/5	4/5
1580	0/5	3/5	3/10
2510	1/5	4/5	5/10
3980	2/5	-	2/5
6310	5/5	-	5/5

* not tested

3. Statistics

The data did not warrant statistical analysis.

ES	*Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

Findings:

A. MORTALITY

Details are provided in Table B.6. 14. No mortalities occurred at 1580 mg / kg for male rats.

Oral LD_{50} for males = 3.81 g/kg bw

for females = 1.28 g/kg bw

B. CLINICAL OBSERVATIONS

Females were more susceptible to Pyrethrum Extract (55,99 %) than males. The main signs for intoxication were increased responsiveness to external stimuli, tremors, salivation and ruffled appearance.

C. BODY WEIGHT

All animals had gained weight 7 and 14 days following dosis.

D. NECROPSY

Gross pathology showed congested lungs (at males from 3980 mg/kg onwards, at females from 1260 mg/kg onwards)

E. DEFICIENCIES

The study was not conducted under GLP, since GLP was not compulsory in 1986, when the study was performed. It was conducted in accordance with generally accepted scientific principles. A NOAL could not be derived from this study.

Conlcusions:

The oral LD_{50} of the test compound was determined to be 3.92 g/kg for males and 1.28 g/kg for females. In accordance with the provisions of Council Directive 67/548/EEC, classification is not required. (Costello, B.A. 1986)

5) (A) Letter: An exploratory 5-day dermal irritation study in New Zealand white rabbits using Pyrethrum Extract; Schoenig G.P., 1991; Cross reference IIA 5.2.4/02

Guidelines:

None

GLP:

No

The study is not acceptable due to absent information about many parameters as purity of the test article, batch number, temperature, humidity and others.

Executive Summary:

Three groups of two rabbits (one male and one female) were used to assess the dermal irritation of Pyrethrum Extract upon repeated application at concentrations of 25%, 50% and 75% in corn oil. The test solution (4.0 mL/kg) was

Explanatory abstract for five consecutive days to the shaven intact skin on the back of each rabbit. After a skin contact period of 6 hours per day, the test article was wiped with tepid tap water and dried with disposable terrycloth (paper) towelling.

Animals were observed twice daily for mortality and moribundity and once daily for dermal observations using the Draize method. No mortalities and no test substance related clinical signs of systemic intoxication or influences on body weights were observed. Daily dermal application of 4 mL of 25, 50 or 75 % Pyrethrum Extract in corn oil for five continuous days caused time- and dosage-dependant formation of slight to moderate erythema to the rabbit.

Material and methods:

A. MATERIALS

1. Test Material:	Pyrethrum extract
Description:	Not stated
Lot/Batch #:	Not stated
Purity:	Not stated
CAS #:	As given in section 2
Stability of test compound:	Not determined
2. Vehicle and/or positive control:	corn oil
3. Test animals	
Species:	Rabbit
Strain:	New Zealand White
Age:	Young adult,
Weight at dosing:	Males: 2868 g-3119 g Females: 2565 g-3425 g
Source:	[redacted] Not recorded
Acclimation period:	Not recorded
Diet:	Not recorded
Water:	Animals were individually housed in labeled cages
Housing:	with perforated floors
Environmental conditions:	
Temperature:	No specification
Humidity:	Not reported
Air changes:	Not reported
Photoperiod:	Not reported

B. STUDY DESIGN AND METHODS

1. In life dates: 18 June to 23 June 1991

2. Animal assignment and treatment

acetum

Three groups of 1 male and 1 female rabbit each were used to assess the dermal irritation of the test substance at dosage concentrations of 25 %, 50 % and 75 % in corn oil (w/v). The test substance was applied once daily for five consecutive days to the shaven intact skin on the back of each rabbit, at a constant dose volume of 4.0 mL/kg. The test substance was covered with a porous gauze dressing fixed with non-irritating tape throughout a 6-h daily exposure period, afterwards the test site was washed with tepid water and dried with disposable terrycloth (paper) towelling.

Animals were observed twice per day for mortality and overt toxicity and daily for irritation effects using the Draize method. Individual body weights were recorded at initiation and at study termination (after 5 days).

Findings:

No mortalities and no test substance related clinical signs of systemic intoxication. No test substance related influence on body weights.

The initiation scores are summarized in Table B.6. 18.

During the first 2 days no erythema were observed except one very slight erythema in the highest concentration group. From day 3 onwards a time and dose dependant increase becomes obvious, from no erythema (grade 0) at one male of the lowest concentration group on day 3 to moderate erythema (grade 3) at four animals of the higher concentration groups on day 5.

No edema were observed at the animals of all test substance concentrations during the first 4 days. One male of the highest concentration group showed very slight edema formation (grade 1) on days 5 and 6.

Daily dermal application of 4 mL 25, 50 or 75 % Pyrethrum Extract in corn oil for five continuous days causes time- and dosage-dependant formation of slight to moderate erythema to the rabbit, whereas the formation of edema generally does not appear (except very slight edema at one male during the end of the test period).

Table B.6. 18: Individual and mean skin irritation scores according to the Draize scheme

Test Substance	**Erythema and Eschar Formation**						**Edema Formation**					
Conc. %	**25 %**		**50 %**		**75 %**		**25 %**		**50 %**		**75 %**	
days	M	F	M	F	M	F	M	F	M	F	M	F
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	1	0	0	0	0	0	0
3	0	1	1	1	2	2	0	0	0	0	0	0
4	1	2	2	2	2	3	0	0	0	0	1	0
5	2	2	3	3	3	3	0	0	0	0	1	0
6	2	2	3	2	3	3						
average score, Draize scores (0 to maximum 4)	0.83	1.2	1.5	1.3	1.67	2.0	0	0	0	0	0	0.3

Conclusions:

Pyrethrum Extract was non-irritant to rabbit skin. On the basis of this study Pyrethrins do not warrant classification as being irritating to the skin. (Schoening, P 1991)

6) Pyrethrum Extract, Range-Finding Teratology Study in Rats; Schardein J.L., 1979a;

Cross reference IIA 5.6.2/01

Guidelines:

None

GLP:

EPA GLP practice standards May 2, 1984

The study is acceptable as supportive information

Executive Summary:

Mated Charles River COBS® CD® female rats, assigned to one control and five treatment groups of 5 animals each, were used in this range-finding study to determine dose levels of pyrethrins for a definitive teratology study. Dose levels of 37.5, 75, 150, 300 and 600 mg/kg bw/day in terms of actual pyrethrin content were administered orally by gavage as a single daily dose on days 6 through 15 of gestation at a volume of 3 mL/kg. The control group received the vehicle only, 0.5% methylcellulose, on a comparable regime. Uterine examinations were performed on all surviving females on gestation day 20.

Treatment-related maternal toxicity, in terms of mortality and convulsions and/or tremors, occurred at 150, 300 and 600 mg/kg bw/day. There was no treatment-related maternal mortality at 75 mg/kg bw/day, although tremors were observed in this group. No treatment-related clinical signs were observed at 37.5 mg/kg bw/day. No dose-related differences from controls were noted in the mean number of viable foetuses, the mean postimplantation loss and the mean number of implantations and corpora lutea of the dams treated with 37.5, 75 and 150 mg/kg/day.

Based on these results, dose levels of 5, 25 and 75 mg/kg bw/day were selected for use in a definitive teratology study.

Materials and methods:

A. MATERIALS

1. Test Material: Pyrethrum Extract Task Force Blend

Description: Amber liquid

Lot/Batch number: #FEK-99, 08/05/86

Purity: 57.574%

CAS#: Not reported

Stability of test compound: 24-hour stability of the test suspension was established

2. Vehicle: 0.5% methylcellulose

3. Test Animals

Species: Rat

Strain: COBS® CD®

Age/weight at receipt: 70-day old, weight at mating 220 to 225 g

Source: Charles River Laboratories, Inc., Portage, Michigan, USA.

Housing: Individually in suspended wire-mesh cages

Acclimatisation period: 12 days

Diet: Purina® Certified Rodent Chow® #5002

Water: Tap Water *ad libitum*

Environmental conditions: Temperature: 22 to 44°C

Humidity: 38 to 92%

12 hours of fluorescent light and 12 hours of darkness provided per 24 hours

B. STUDY DESIGN

1. In-life dates: 3 to 26 September 1986

2. Mating procedure

At the end of the acclimation period, all animals were weighed and subjected to a detailed physical examination. At this time, animals considered suitable for study were cohabited with stock males used exclusively for this purpose. One female and one male rat of the same strain and source were placed together for mating. The occurrence of copulation was determined by daily inspection for a copulatory plug. The day evidence of mating was detected was designated day 0 of gestation and the female was returned to an individual cage, assigned a permanent animal number and properly identified by ear tag.

3. Animal assignment

Mated females were consecutively assigned in a block design to one control and five treatment groups consisting of 5 rats each by the following procedures. The order in which the mated females were assigned corresponded to the day of the copulatory plug was observed and the order in which the animal appeared on the breading record. The first mated female on the breading record was assigned to the first group, the second mated female assigned to the second group; all remaining animals were assigned in this manner until the required number of mated females had been placed into each group.

Table 6.6.2/01-1 Number of animals and treatment groups

Group no.	Females/group	Treatment	Dose level (mg/kg bw/day)	Dose concentration (mg/mL)	Dose volume (mL/kg bw)
1	5	Vehicle	0	0	3
2	5	Actual pyrethrins	37.5	12.5	3
3	5		75	25	3
4	5		150	50	3
5	5		300	100	3
6	5		600	200	3

4. Dosage preparation and analysis

The appropriate amounts of pyrethrum extract for each group was suspended in the vehicle, 0.5% methylcellulose, using a tissue homogenizer. A correction factor of 1.7369 was used in test article calculations to adjust for the purity of the test article. The required amount of additional vehicle was then added to this suspension and the resulting mixture was shaken

by hand. The test article was prepared daily (with two exceptions) at concentrations to permit the administration of dose levels of 37.5, 75, 150, 300 and 600 mg/kg bw/day at a dose volume of 3 mL/kg. The suspensions administered on occasions designated for sample collection were prepared the day before to allow time for analysis prior to administration. The 300 and 600 mg/kg bw/day dosing solutions were no longer prepared after the surviving females in these groups were humanely killed due to the occurrence of excessive toxicity at these dose levels.

Replicate samples were collected on approximately the first and last days of administration from the top, middle and bottom of the dosing suspensions and analysed for homogeneity and concentration of the active ingredient (pyrethrin) of the test article. An additional sample of each dosing suspension was collected on these same days and stored frozen for possible future analysis.

5. Dose administration

The test article was administered as a single daily dose in vehicle 0.5% methylcellulose, on days 6 through 15 of gestation. The prepared test article suspensions were stirred using a magnetic stir bar and stir plate during administration. Animals were dosed by oral gavage using a 1 cc glass syringe and 16-gauge, 7.6 cm long stainless-steel dosing needle. A constant dose volume of 3 mL/kg body weight was used, adjusted to the most recent body weight. The control group received the vehicle only on a comparable regimen.

C. METHODS

1. Maternal observations

Throughout the study, the animals were observed twice daily for mortality and overt changes in appearance and behaviour. The presence and duration of clinical signs of toxicity were recorded once daily on days 6 through 20 of gestation. Females not surviving to scheduled sacrifice were necropsied in an attempt to determine the cause of death.

Due to the occurrence of excessive toxicity in the dosed females at 300 and 600 mg/kg bw/day, all surviving animals at these doses were humanely killed on gestation days 5 or 6. No necropsy examinations were conducted on these animals.

2. Body weight

Individual body weights were recorded on gestation days 0, 6, 9, 12, 16 and 20.

3. *Post Mortem* Investigations

On gestation day 20 all females were sacrificed by carbon dioxide inhalation. Immediately following sacrifice, the uterus and ovaries were exposed by an abdominal incision and the number and location of the viable and non-viable foetuses, early and late resorptions and the total number of implantation and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes and the carcases discarded. Uteri from females that appeared non-gravid were opened and placed in 10% ammonium sulphide solution for detections of implantations.

4. Statistical analyses

Statistical analysis of data was not conducted.

Results and discussion:

Concentration analysis results

Analytical results of the periodic test article: 0.5% methylcellulose suspensions, approximately the first and last days of administration, contained mean concentrations

ranging from 91 to 102% of target levels.

Homogeneity and stability results

Stability analysis showed that pyrethrum extract was stable for up to 24 hours in 0.5% methylcellulose suspensions under normal laboratory conditions. Homogeneity evaluation of pyrethrin in 0.5% methylcellulose showed mean homogeneity assay values for all groups analysed ranging from 91 to 102% of target concentration. These results indicate that a homogeneous test suspension was produced.

A. OBSERVATIONS

1. Mortality and maternal clinical signs of toxicity

Two, three and two females died post-dose on gestation day 6 or 7 in the 150, 300 and 600 mg/kg/day dose groups, respectively. Convulsions, and tremors in some instances, were observed post-dose on the day of death for these females. As a result of the toxicity observed in these groups, the surviving females in the 300 and 600 mg/kg bw/day groups were humanely sacrificed.

Tremors and/or convulsions were observed post-dose for two dams at 75 mg/kg bw/day, and in two rats which survived at 150 mg/kg bw/day. Wet red material around the nose and eyes was also noted postdose for one of these females at 150 mg/kg bw/day. There were no treatment-related differences in the appearance or behaviour of the rats at 37.5 mg/kg bw/day.

Table 6.6.2/01-2 Summary of clinical observations

Observation	**Pyrethrin Dose level (mg/kg bw/day)**					
	0	**37.5**	**75**	**150**	**300**	**600**
Number of animals observed	5	5	5	5	4[a]	3[b]
No visible abnormalities	4	3	3	3	3	2
Died				2	3	2
Killed (due to excessive toxicity at this dose)					2[a]	3[b]
Hair loss	1	2	2		1	1
Tremors (post-dose)			2	3	1	
Convulsions (post-dose)				4	3	3
Material around nose and eyes, wet, red (post-dose)				1		

[a] One female sacrificed gestation day 5, prior to initiation of antemortem observations

[b] Two females sacrificed gestation day 5, prior to initiation of antemortem observations

B. BODY WEIGHT AND BODY WEIGHT GAIN

As a result of mortality and resultant early termination, the body weight gain of the 300 and 600 mg/kg bw/day dams could not be assessed during treatment. There were no treatment-related differences in body weight of the dams at 37.5, 75 and 150 mg/kg bw/day, during the treatment period (gestation days 6 through 15) or during the entire gestation period (gestation days 0 to 20).

Table 6.6.2.1-3 Intergroup comparison of group mean bodyweight gain (g)

Day	**Pyrethrins (mg/kg bw/day)[a, b]**					
	0	**37.5**	**75**	**150**	**300**	**600**
0 to 6	34	36	39	37	40	29

6 to 9	6	7	4	7	--	--
9 to 12	14	16	17	13	--	--
12 to 16	22	24	21	22	--	--
16 to 20	62	52	55	57	--	--
6 to 15[c] (treatment period)	42	48	41	42	--	--
0 to 20	138	137	135	133	--	--

[a] Non-gravid animals were not included in calculation of means
[b] Values represent the mean of the individual changes in maternal body weight for these intervals
[c] Gestation day 16 values were utilised to reflect the entire 10-day treatment period
-- Not applicable

C. FOOD CONSUMPTION

Food consumption was not recorded.

D. SACRIFICE AND PATHOLOGY

1. Gross pathology

No gross lesions were present at gross necropsy for the animals that died. No gross lesions were observed for the control and treated animals at necropsy.

2. Caesarean section data

Gestation day 20 uterine observation data was not available for the females in the 300 and 600 mg/kg/day dosage groups, since these groups were terminated early in the gestation period.

No dose-related differences were noted in the mean number of viable foetuses, the mean postimplantation loss and the mean number of implantations and corpora lutea of dams at 37.5, 75 and 150 mg/kg bw/day, in comparison to the control values.

Table 6.6.2/01-4 Caesarean section observations for all pregnant females

Observation	**Pyrethrins (mg/kg bw/day)**					
	0	**37.5**	**75**	**150**	**300**	**600**
Animals Assigned (Mated)	5	5	5	5	5	5
Animals that were gravid	5	5	4	5	1	0
Animal that died	0	0	0	2	3	2
Nongravid	--	--	--	0	2	2
Gravid	--	--	--	2	1	0
Humanely killed (not examined)	--	--	--	--	2	3
Animals examined at uterine examination	5	5	5	3	0	0
Nongravid	0	0	1	0	--	--
Gravid	5	5	4	3	--	--
Dams with resorptions only	0	0	0	0	--	--
Dams with viable foetuses	5	5	4	3	--	--
Viable foetuses/dam	14.4	12.6	13.0	14.0	--	--
Post-implantation loss/dam	0.4	2.2	2.0	0.3	--	--
Total implantations /dam	14.8	14.8	15.0	14.3	--	--
Corpora lutea/dam	15.8	16.0	16.0	15.0	--	--
Group mean preimplantation loss (%)[a]	6.3	7.5	6.3	4.4	--	--
Group mean post-implantation loss (%)[b]	2.7	14.9	13.3	2.3	--	--

[a] Total number of corpora lutea- Total number of implantations x 100
Total Number of corpora lutea
[b] Total number of implantations- Total number of viable foetuses x 100
Total Number of implantations

-- Not applicable

Conclusion:

Treatment-related maternal toxicity, in terms of convulsions and/or tremors and mortality, occurred at 150, 300 and 600 mg/kg bw/day. No excessive dose-related maternal mortality was evident at 75 mg/kg bw/day, although tremors were observed in this group. No treatment related clinical signs were observed at 37.5 mg/kg bw/day.

Based on these results, dose levels of 5, 25 and 75 mg/kg bw/day were selected for use in the definitive teratology study.

A. ASSESSMENT AND CONCLUSION BY APPLICANT

Assessment:

This non-guideline range-finding study is supportive information.

Conclusion:

Provides justification for the dose levels selected in the definitive study.

B. ASSESSMENT AND CONCLUSION BY RMS

This non-guideline range-finding study in rat is considered appropriate for the intended aim of determine a dose levels of pyrethrins for a definitive teratology study.

On the basis of the results from this study, dose levels of 5, 25 and 75 mg/kg bw/day are considered suitable for use in the definitive teratology study.

Please note that for this study analytical methods for current standards are o longer considered acceptable (please refers to Vol 3 CA B.5). Anyway this study is considered fit for pourpose, pending on conclusion on storage stability issues.

7) Pyrethrum extract Range-Finding Teratology Study in Rabbits; Schardein J.L., 1979b;

Cross reference IIA 5.6.2/02

Guidelines:

None

GLP:

EPA GLP practice standards May 2, 1984

The study is acceptable as supportive information

Executive Summary:

Inseminated female New Zealand White SPF rabbits randomly assigned to one control and five treatment groups of 5 animals each were used in this range-finding study to determine dose levels of pyrethrins for a teratology study. Dose levels of 37.7, 75, 150, 300 and 600 mg/kg bw/day in terms of actual pyrethrin content were administered orally by gavage as a single daily dose on days 7 through 19 of gestation at a volume of 3 mL/kg. The control group received the vehicle only, 0.5% methylcellulose, on a comparable regimen. Uterine examinations were performed on all surviving females on gestation day 29. Treatment-related maternal toxicity in terms of mortality, tremors/convulsions and weight loss and foetotoxicity in terms of high post-implantation loss was observed at 600 mg/kg bw/day. Maternal toxicity in terms of weight loss during the treatment period and tremors were

evident at 300 mg/kg bw/day. No clear treatment-related effects were observed at 37.5, 75 or 150 mg/kg bw/day. Based on these results, dose levels of 25, 100 and 250 mg/kg bw/day were selected for the definitive teratology study.

Materials and methods:

A. MATERIALS

1. Test Material: Pyrethrum Extract Task Force Blend

Description: Amber liquid

Lot/Batch number: #FEK-99, 08/05/86

Purity: 57.574%

CAS#: Not reported

Stability of test compound: 24-hour stability of the test suspension was established

2. Vehicle: 0.5% methylcellulose

3. Test Animals

Species: Rabbit

Strain: New Zealand White SPF

Age/weight at receipt: 4-month old/3160 to 4132 g on gestation day 0

Source: Hazleton Research Animals, Denver, Pennsylvania, USA.

Housing: Individually in wire cages with Deotized animal cage board waste pan litters

Acclimatisation period: 48 days

Diet: Purina® Certified Rabbit Chow® #5322 *ad libitum*

Water: Tap Water *ad libitum*

Environmental conditions: Temperature: 23 to 26°C

Humidity: 36 to 78%

12 hours of fluorescent light and 12 hours of darkness provided per 24 hours

B. STUDY DESIGN

1. In-life dates: 2 September to 1 October 1986.

2. Mating procedure

The females were approximately 5 ½ months old at the time of insemination and weighed

between 3160 and 4132 g on gestation day 0. Approximately three weeks prior to insemination, the does were superovulated by an injection of 50 U.S.P. units of human chorionic gonadotropin via a marginal ear vein. Semen was collected from five proven male rabbits of the same breed and source. Semen was collected using an artificial vagina and the gelatinous plug was removed from the ejaculate. The semen was immediately evaluated for motility and was used for insemination only if the motility was 60% or greater, as assessed subjectively. The ejaculate was diluted with 3.0 mL of 0.9% sodium chloride for injection, U.S.P., at 33-37°C and 0.3 mL of this dilute semen was introduced into the anterior vagina of the female using an insemination pipette. Immediately after insemination, ovulation was induced by an injection of 100 U.S.P. units of human chorionic gonadotropin into the marginal ear vein. Semen from one male was used to inseminate an equal number of females in each group. Insemination procedures were performed on one day. The day of insemination was designated as day 0 of gestation.

3. Animal assignment

At the end of the acclimation period, all animals were weighed and subjected to a detailed physical examination. Animals considered suitable for study were randomly assigned to one control group and five treatment groups of 5 rabbits each using a weight stratified randomisation procedure. Bartlett's test for homogeneity of variance was applied to the groups; the groups were judged to be homogeneous.

Table 6.6.2/02-1 Number of animals and treatment groups

Group no.	Females/group	Treatment	Dose level (mg/kg bw/day)	Dose concentration (mg/mL)	Dose volumen (mL/kg bw)
1	5	Vehicle	0	0	3
2	5	Actual pyrethrins	37.5	12.5	3
3	5		75	25	3
4	5		150	50	3
5	5		300	100	3
6	5		600	200	3

4. Dosage preparation and analysis

The appropriate amount of pyrethrum extract for each group was suspended in the vehicle (0.5% methylcellulose) using a tissue homogeniser. A correction factor of 1.7369 was used in test article preparation calculations to adjust for the purity of the test article (57.574%). The required amount of additional vehicle was then added to this suspension and the resulting mixture was shaken by hand. The test article was prepared daily (with two exceptions) at concentrations to permit the administration of dose levels of 37.5, 75, 150, 300 and 600 mg/kg bw/day at a dose volume of 3 mL/kg. The suspensions administered on gestation days 7 and 15 were designated to be analysed for pyrethrin concentration and were prepared the day before to allow time for analysis prior to administration.

Stability analysis

Prior to initiation of the test article administration period, the 24-hour stability of pyrethrins in suspensions was assessed. In addition, a sample of each dosing solution was collected and frozen for possible future analysis.

Homogeneity and pyrethrin concentration analysis

Replicate samples were collected from preparations administered on gestation days 7 and 15, from the top, middle and bottom of the dosing suspensions and analysed for the homogeneity and concentration. An additional sample of each dosing suspension was collected at the same time and frozen for possible future analysis.

5. Dose administration

The dosing preparations were administered as a single daily dose on days 7 through 19 of gestation. The dosing preparation suspensions were stirred using a magnetic stir bar and stir plate during administration. The dosing preparations were administered by intragastric intubation using 35 cc disposable plastic (first two days of administration) or glass (remainder of administration period) syringes and 12-gauge, 15 cm long curved stainless-steel dosing needles. The control group received the vehicle only on a comparable regimen at a volume of 3 mL/kg. Individual doses were determined from the most recently recorded individual body weights.

C. METHODS

1. Maternal observations

Throughout the study, the animals were observed twice daily for mortality and overt changes in appearance and behaviour. The presence and duration of clinical signs of toxicity were recorded once daily on days 7 through 29 of gestation, although detailed observations recorded on days 20 through 29 were not required by protocol. Females not surviving to the scheduled sacrifice were necropsied in an attempt to determine the cause of death. Any female showing signs of abortion or premature delivery was sacrificed and necropsied on the day such evidence was observed and the aborted tissue was examined and discarded.

2. Body weight

Individual maternal body weights were recorded on gestation days 0, 7, 13, 20, 24 and 29.

3. *Post Mortem* Investigations

On gestation day 29, all surviving females were sacrificed by an injection of sodium pentobarbital via a marginal ear vein. Immediately following sacrifice, the uterus and

ovaries were exposed by an abdominal incision and the number and location of the viable and non-viable foetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes and the carcases discarded. Maternal tissues were preserved in neutral buffered 10% formalin for possible histopathological examination as deemed necessary by the gross findings. Uteri from females that appeared non-gravid were opened and placed in 10% ammonium sulphide solution for detections of implantations.

Results and discussion:

Test article stability in 0.5% methylcellulose

Stability analysis showed that pyrethrum extract was stable for up to 24 hours in 0.5% methylcellulose suspensions under normal laboratory conditions.

Concentration analysis results

Test suspensions prepared for administration on gestation days 8 and 19 at 25, 100 and 250 mg/kg bw/day contained 90 to 104% of the target concentrations (means of assay values).

Homogeneity and stability results

Pyrethrin concentrations found in samples taken from the top, middle and bottom of the 25, 100 and 250 mg/kg bw/day dosing suspensions prepared for administration on gestation days 8 and 19 were within ±5% of the mean values. The dosing suspensions were homogeneous. Stability was also confirmed, as average levels at 24 hours ranged from 94 to 100% of the average zero hours concentrations.

E. OBSERVATIONS

1. Mortality

One doe died at 37.5 mg/kg bw/day and two does died at 600 mg/kg bw/day.

2. Maternal clinical signs of toxicity

No visible abnormalities were noted prior to death on gestation day 20 for the doe at 37.5 mg/kg bw/day. Findings for the does that dies on gestation days 18 and 20 at 600 mg/kg bw/day included tremors, convulsions, laboured breathing, hunched posture, excessive salivation and/or a wet, yellow-stained area around the mouth and nose. With the exception of the last finding, these pharmacotoxic signs genrally occurred post-dose.

One doe at 37.5 mg/kg bw/day and one doe at 300 mg/kg bw/day aborted on days 22 and 25 of gestation, respectively. One doe at 600 mg/kg bw/day delivered on gestation day 29. Antemortem findings noted for these animals included emaciation (600 mg/kg bw/day), tremors (300 and 600 mg/kg bw/day), nasal discharge post-dose (300 mg/kg bw/day) and dark brown liquid in the cage pan (37.5 mg/kg bw/day).

Tremors, and convulsions in some cases, occurred in all the females at 600 mg/kg bw/day. Tremors also occurred in one female at 300 mg/kg bw/day. There were no treatment-related differences in appearance and behaviour of does at 37.5, 75 and 150 mg/kg bw/day.

F. BODY WEIGHT AND BODY WEIGHT GAIN

Dose-related mean body weight losses occurred for does at 300 and 600 mg/kg bw/day during the treatment period (gestation days 7 through 19). Similarly weight loss was noted for does at 600 mg/kg bw/day over the entire gestation period. Reduced weight gain relative to control was observed in dams at 300 mg/kg bw/day over the entire gestion period.

There were no consistent treatment-related differnces in body weight gain in does at 37.5, 75 and 150 mg/kg bw/day, in comparison to control.

Table 6.6.2/02-2 Intergroup comparison of group mean bodyweight gain (g)

Day	Pyrethrins (mg/kg bw/day)[a]					
	0	**37.5**	**75**	**150**	**300**	**600**
0 to 7	157	157	172	206	203	141
7 to 13	47	105	-24	31	-94	-299
13 to 20	114	117	20	105	-121	-325
20 to 24	-21	-89	47	0	26	115
24 to 29	-39	-185	10	22	41	60
7 to 19[b] (treatment period)	161	219	-4	136	-215	-514
0 to 29	258	266	226	365	160	-56

[a] Values represent the mean of the individual changes in maternal body weight for these intervals
[b] Gestation day 20 values were utilised to reflect the entire 10-day treatment period

G. FOOD CONSUMPTION

Food consumption was not recorded.

H. SACRIFICE AND PATHOLOGY

1. Gross pathology

There were no noteworthy maternal necropsy findings. There were no apparent treatment-related gross pathological changes in animals sacrificed at the end of the study, and for those does that aborted or those that died.

2. Caesarean section data

There was an increase in mean post-implantation loss and a resultant decrease in the mean number of viable foetuses at 600 mg/kg bw/day, relative to control. Slight increases in post-implantation loss were also recorded at 150 and 300 mg/kg bw/day; however, it was unclear if the magnitude of increase was of biological significance. No treatment-related differences in the values for the uterine examination parameters were noted at 37.5 and 75 mg/kg bw/day.

Table 6.6.2/02-3 Caesarean section observations for all pregnant females

Observation	Pyrethrins (mg/kg bw/day)					
	0	**37.5**	**75**	**1510**	**300**	**600**

Animals Assigned (Mated)	5	5	5	5	5	5
Animals that were gravid	5	5	5	5	5	4
Animals that died	0	1	0	0	0	2
Nongravid	-	0	-	-	-	0
Gravid	-	1	-	-	-	2
Animal that aborted/delivered	0	1	0	0	1	1
Animals examined at uterine examination	5	3	5	5	4	2
Nongravid	0	0	0	0	0	1
Gravid	5	3	5	5	4	1
Does with rerptions only	0	0	0	0	0	0
Does with viable foetuses	5	3	5	5	4	1
Viable foetuses/doe	6.8	8.0	8.6	7.0	7.0	5.0
Post-implantation loss/doe	0.2	0.0	0.2	1.4	1.0	4.0
Total implantations/doe	7.0	8.0	8.8	8.4	8.0	9.0
Corpora lutea/doe	12.0	12.3	11.2	12.4	11.5	15.0
Group mean pre-implantation loss (%)[a]	41.7	35.1	21.4	32.3	30.4	40.0
Group mean post-implantation loss (%)[b]	2.9	0.0	2.3	16.7	12.5	44.4

[a] Total number of corpora lutea- Total number of implantations x100

Total Number of corpora lutea

[b] Total number of implantations- Total number of viable foetuses x100

Total Number of implantations

- Nota applicable

Conclusion:

Treatment-related maternal toxicity in terms of mortality, tremors/convulsions and weight loss and foetotoxicity in terms of high post-implantation loss were observed at 600 mg/kg bw/day. Maternal toxicity in terms of weight loss during the treatment period and tremors was evident at 300 mg/kg bw/day. No clear treatment-related effects were observed at 37.5, 75 or 150 mg/kg bw/day.

Based on these results, dose levels of 25, 100 and 250 mg/kg bw/day were considered appropriate for the definitive teratology study in rabbits.

A. ASSESSMENT AND CONCLUSION BY APPLICANT

Assessment:

This non-guideline range-finding study is supportive information.

Conclusion:

Provides justification for the dose levels selected in the definitive study.

B. ASSESSMENT AND CONCLUSION BY RMS

This non-guideline range-finding study in rabbit is considered appropriate for the intended aim of determine a dose levels of pyrethrins for a definitive teratology study in rabbits.

On the basis of the results from this study, dose levels of 25, 100 and 250 mg/kg bw/day are considered suitable for use in the definitive teratology study.

Please note that for this study analytical methods for current standards are no longer considered acceptable (please refers to Vol 3 CA B.5). Anyway this study is considered fit for pourpose, pending on conclusion on storage stability issues.

8) Peroral (Gavage) Neurotoxicity Probe Study with Pyrethrum Extract in CD® Rats;

Hermansky S.J. & Hurley J.M., 1993a; Cross reference IIA 5.7.1/01

Guidelines:

None

GLP:

Yes, conducted under GLP/Officially recognised testing facilities

The study is acceptable as supportive information

Executive Summary:

The probe study was divided into 2 phases. In Phase I, 1 male and 1 female rat/dose, were dosed by oral gavage, over a broad range of doses to generate preliminary information with which to select doses for more extensive testing in Phase II. In Phase I, clinical signs of toxicity were monitored hourly for several hours after dosing. In Phase II, groups of 2-4 rats/sex/group were employed and clinical signs of toxicity were recorded and specific evaluations for tremors, arousal state, and gait were made at least hourly for several hours and 24 hours after dosing.

Phase I

Males were treated with a solution of pyrethrum extract in corn oil, prepared to contain 25% total pyrethrins, at dose levels of 0.2, 0.4, 0.8, 1.4, and 2.5 g total pyrethrins/kg body weight. Animals treated with 1.4 and 2.5 g/kg, died within 5.5 hours of dosing. Tremors were observed at all dose levels within 2 hours after dosing. Tremors persisted through the 10-hour observation period for the male at 0.8 g/kg and through the 4-hour observation for the male at 0.4 g/kg. Tremors for the male at 0.2 g/kg were not observed after the 2-hour observation. Other observations recorded prior to death for the 1.4 and 2.5 g/kg animals included lying on stomach, labored respiration, salivation, urine stains, and prostration.

A dosing solution containing 10% total pyrethrins was selected for females in Phase I based on the results obtained for males during Phase I and LD50 information supplied by the Sponsor indicating that females were more sensitive to the test substance than males. Females were treated at dose levels of 0.05, 0.1, 0.2, 0.4, and 0.8 g total pyrethrins/kg body weight. Dose levels of 0.2, 0.4, and 0.8 g/kg resulted in death within 5 hours of dosing, in addition, tremors were recorded at all dose levels within 2 hours of dosing. Tremors persisted through the 3-hour observation period for the female at 0.1 g/kg.

Tremors for the female at 0.05 g/kg were not observed after the 2-hour observation. Other observations recorded prior to death for the 0.4 and 0.8 g/kg animals were like those observed for males.

Phase II

Males were administered a solution of pyrethrum extract in corn oil at a concentration of 10% total pyrethrins at dose levels of 0.0, 0.04, 0.1, 0.2, and 0.4 g total pyrethrins/kg body weight. Animals in the control group were administered corn oil at a volume equal to the volume of test solution administered to the highest dose group. No mortality was observed. Tremors were observed in males dosed at 0.4 g/kg from the 2- through the 5-hour observation periods. No tremors were recorded for males in any other dose group at any observation period or in any animals at 24 hours after dosing. Salivation was observed for 1 animal in the 0.2 g/kg group. A possible decrease in the level of arousal compared to control was observed in the 3 highest dose groups at the 5- and 24-hour observation periods. The time to peak effect in the 0.4 g/kg group was estimated to be between 3 and 5 hours after administration.

In Phase II, females initially were treated with a solution of pyrethrum extract in corn oil at a concentration of 2.5% total pyrethrins at dose levels of 0.0, 0.025, 0.05, 0.1, and 0.15 g total pyrethrins/kg body weight. Animals in the control group were administered corn oil at a volume equal to the volume of test solution administered to the highest dose group. No females died at these dose levels. Clinical signs were limited to the 0.15 g/kg group and included fine tremors for 1 female 6 and 7 hours after treatment and altered gait for another female at the 2-hour through 24-hour observation periods. Because of the limited number of clinical signs observed in these females, 12 additional females (2/dose level) were treated with a solution of pyrethrum extract in corn oil at a concentration of 5% total pyrethrins at dose levels of 0.0, 0.025, 0.05, 0.1, 0.15, and 0.2 g total pyrethrins/kg body weight. Animals in the control group were administered corn oil at a volume equal to the volume of test solution administered to the highest dose group. Tremors were observed for both female animals dosed at 0.2 g/kg and 1 of 2 females in each of the 0.15 and 0.1 g/kg groups. These tremors were generally described as fine and were observed for all 3 of these groups during the 3 and 4-hour observation periods and for the highest dose group during the 2, 5, and 6-hour observation periods. One animal from the 0.2 g/kg group was also described as hyperactive and as having perinasal encrustation. The time to peak effect was estimated to be between 3 and 5 hours after dose administration. Based upon the results of this study, dose levels of 0.04, 0.125, and 0.4 g total pyrethrins/kg body weight were selected for males in the definitive acute neurotoxicity study. These doses were to be administered as a solution of pyrethrum extract prepared in corn oil to contain 10% total pyrethrins. For females, dose levels of 0.02, 0.063, and 0.2 g total pyrethrins/kg body weight were selected for use in the definitive acute neurotoxicity study. These doses were to be administered as a solution of pyrethrum extract prepared in corn oil to contain 5% total pyrethrins. Based upon the time course of effects observed in this study, 3 to 5 hours was selected as the time for the first post-exposure evaluation in the definitive acute neurotoxicity study.

Material and methods:

A. MATERIALS

1. Test Material: Pyrethrum Extract

Description: Dark viscous liquid

Lot/Batch: LS92-37, Task Force Blend FEK-99

Purity: 57.467% (w/w)

2. Vehicle: Mazola corn oil

3. Test Animals

Species: Rat

Strain: Charles River CD® (Sprague-Dawley)

Age: 35 days

Sex: Male and female

Weight at dosing:

Source: Charles River Laboratories (Portage, MI)

Acclimation period: Approximately 15 days

Diet: Ground, certified Rodent chow® #5002 (Purina Mills, Inc.) *ad libitum*

Water: Tap water *ad libitum*

Housing: Individually housed

Environmental Conditions: Temperature: 66-77 ºF
Humidity: 40-70%
Photoperiod: 12-hour light/dark cycle

B. STUDY DESIGN

1. In-life dates: March 1992 to April 1992

2. Animal assignment and treatment

Forty male and 40 female CD rats were assigned to the study. In phase I, 1 male and 1 female rat was assigned to each treatment group. For the first part of phase II, groups of 4 rats/sex/group were assigned to treatment and control groups using a weight stratified computerized randomization procedure. The animals selected for use in phase I of the study were randomly selected from the remaining animals after this randomisation procedure. For the second part of phase II, female animals not selected for either Phase I or phase II were assigned to 5 dose groups so that each group contained an animal with a high body weight and an animal with a low body weight. Two female control animals from the first part of phase II were selected for use as a control group in the second part of phase II.

In phase I, males were treated with a 25% total pyrethrins solution in corn oil, at dose levels of 0.2, 0,4, 0.8, 1.4, and 2.5 g total pyrethrins/kg bw and females were treated with corn oil solution that contained 10% total pyrethrins at dose levels between 0.05, 0.1, 0.2, 0.4 and 0.8 g total pyrethrins/ kg bw.

In phase II, a dosing solution containing 10% total pyrethrins in corn oil and dose levels of

0.0, 0.04, 0.1, 0.2, and 0.4 g total pyrethrins/kg bw were selected for males. Females initially were treated with a solution of 2.5% pyrethrins in corn oil and doses 0.0, 0.025, 0.05, 0.1, and 0.15 g total pyrethrins/kg bw. Due to the limited number of clinical signs observed in these females, 12 additional females (2/dose level) were treated 5% total pyrethrins in corn oil at dose levels of 0.0, 0.025, 0.05, 0.1, 0.15, and 0.2 g. Animals of the control group were treated with corn oil at a volume equivalent to that administered to the rats in the high dose treatment groups.

Table 6.7.1/01-1 Study Design

Study phase	Dose preparation concentration (% total pyrethrins in corn oil)		Dose/animal (g/kg bw)		Animals assigned	
	Male	Female	Male	Female	Male	Female
I	25%	10%	0.2	0.05	1	1
			0.4	0.1	1	1
			0.8	0.2	1	1
			1.4	0.4	1	1
			2.5	0.8	1	1
II	10%	2.5%	0	0	4	4
			0.04	0.025	4	4
			0.10	0.05	4	4
			0.20	0.10	4	4
			0.40	0.15	4	4
	--	5%	--	0	--	2*
			--	0.025	--	2
			--	0.05	--	2
			--	0.10	--	2
			--	0.15	--	2
			--	0.20	--	2

* Two female control animals from the first part of phase II were selected for use as a control group in the second part of phase II.

3. Dose preparation

At least 2 hours prior to preparation of dosing solutions, the test substance was removed from the refrigerator and equilibrated to room temperature and mixed vigorously by manual inversion for at least 2 minutes. Due to light degradation of the test substance, all solutions of pyrethrum extract were prepared in a photographic dark room in flasks covered with black electrical tape. The dosing solutions were prepared to contain 5% and 10% total pyrethrins by diluting the appropriate amount of test substance in corn oil. Each dosing solution was mixed for a minimum of 15 minutes on a magnetic stir plate. Each dose solution was prepared once, stored refrigerated, and used during a single day of dosing.

4. Statistics

The data for quantitative continuous variables were intercompared for the 3 treatment groups and the control group by use of Levene's test for equality of variances, ANOVA, and t-tests. Incidence data were compared using the Fisher's Exact Test. Incidence data for select FOB endpoints with ordered severity scores were analysed for group differences using Gamma, Kendall's Tau-B, Stuart's Tau-C, and Somers' D measures of association. A nested analysis of motor activity data was performed using repeated measures analysis of variance with dose as the grouping factor and test period and test session time as within subject factors. The epsilon-adjustment procedure (Greenhouse-Geisser correction) was used in the repeated measures analysis of motor activity data. All statistical analysis, except neuropathology frequency comparisons, were performed using BMDP statistical software. For all statistical tests the probability value of <0.05 (two-tailed) was used as the critical level of significance.

C. METHODS

1. Observations

All animals assigned to the study were observed for mortality twice each day, 7 days a week. Clinical signs of overt toxicity were documented once each day. Observations for clinical signs of toxicity or changes in behaviour were made approximately every hour, for several hours following dosing, during all phases of the study. Animals were observed, specifically, for signs of gait alteration, level of arousal, and tremors during phase II of the study.

2. Body weight

Body weights were recorded in the morning prior to dosing.

Results and discussion:

A. OBSERVATIONS

1. Clinical observations

In phase I, tremors were observed in all males within 2 hours of treatment. The tremors were described as severe at various time periods for the animals dosed at the 0.8, 1.4, and 2.5 g/kg dose levels. Tremors were downgraded to slight 9 and 10 hours after dosing for the animals treated with 0.8 g/kg. Additional observations recorded prior to death included lying on the stomach, labored respiration, salivation, urine stains, and prostration. Tremors were not observed after the 2-hour observation for the male dosed at 0.2 g/kg or after the 4-hour observation for the male dosed at 0.4 g/kg.

Tremors were observed in all female animals within 2 hours of treatment. Tremors were not noted in the female dosed at 0.05 g/kg after the 2-hour observation or in the female dosed at 0.1 g/kg following the 3-hour observation. The tremors for the animals dosed at 0.2, 0.4, and 0.8 g/kg were described as coarse and considered to be severe at several observation periods. The female dosed at 0.8 g/kg was observed lying on her stomach and salivating at the 3-hour observation and prostrate with fine tremors, salivation, and diarrhea at the 4-hour observation.

In phase II, effects for the males were largely limited to the high dose group (0.4 g/kg). Fine tremors were observed in 2 of 4 male animals dosed at 0.4 g/kg at the 2-hour observation period. At the 3- and 4-hour observations, all males in this group had tremors (3 of 4 animals had fine tremors and the remaining animal had coarse tremors). Five hours after treatment, fine tremors were observed in 3 of these 4 animals. No tremors were observed at the 24-hour observation period for these animals. Other signs of toxicity in the 0.4 g/kg dose group included piloerection in 1 animal 2 hours and another animal 3 hours after treatment, red extremities in 1 animal 4 and 5 hours after treatment, and perinasal encrustation for 2 animals 3 hours after treatment and for 1 of these animals 4 hours after treatment. Other observations recorded for male animals included salivation for 1 animal in the 0.2 g/kg dose group and perinasal encrustation for l control animal from the 3-hour to the 24-hour observation periods.

A possible decrease in arousal was observed in animals in the 3 highest dose groups at 5 and 24 hours after treatment. All control animals were described as active and alert, while at least 2 of the 4 animals in the 3 highest dose groups were described as inactive and alert.

In the first part of phase II, observations were limited to the females in the highest dose group (0.15 g/kg). Fine tremors were observed in 1 female animal at the 6- and 7-hour observation periods and gait alteration described as "walks on toes" was observed for 1 animal from the 2-hour through the 24-hour observation period. No other observations were recorded for any other female animals in any dose group. In Part 2 of Phase II,

tremors were observed for both females at the highest dose (0.2 g/kg) and l female each at 0.15 and 0.1 g/kg. These tremors were generally described as fine and were observed for all 3 groups during the 3 and 4-hour observation periods and for the highest dose group during the 2-, 5-, and 6-hour observation periods. One animal in the 0.2 g/kg dose group was hyperactive at the 2-hour through 5-hour observation periods and again at the 24-hour observation period and as having perinasal encrustation at the 4-hour observation.

2. Mortality

In phase I, the males dosed at 1.4 and 2.5 g/kg were found dead within 5.5 hours after dosing. The female dosed at 0.2 g/kg was found dead 4 hours after dosing. No observations, other than tremors, were observed for this animal. The females dosed at 0.4 and 0.8 g/kg were found dead 5 hours after dosing.

No mortality was observed during phase II of the study.

B. BODY WEIGHT

No treatment-related changes in bw were observed in males or females in phase II of the study.

Table 6.7.1/01-2 Summary of observations in phase II part one

Group (g/kg)	Males					Females				
	0.000	**0.040**	**0.100**	**0.200**	**0.400**	**0.000**	**0.025**	**0.050**	**0.100**	**0.150**
1 Hour post-treatment										
Active/alert	4	4	4	3	4	4	4	4	4	4
Inactive/alert	0	0	0	1	0	-	-	-	-	-
Tremors (none)	4	4	4	4	4	4	4	4	4	4
2 Hour post-treatment										
Active/alert	4	4	4	3	4	4	4	3	4	4
Inactive/alert	0	0	0	1	0	0	0	1	0	0
Tremors (none)	4	4	4	4	2	4	4	4	4	4
Tremors (fine)	0	0	0	0	2	-	-	-	-	-
3 Hour post-treatment										
Active/alert	4	2	4	3	4	3	4	3	4	3
Inactive/alert	0	2	0	1	0	1	0	1	0	1
Tremors (none)	4	4	4	4	0	4	4	4	4	4
Tremors (fine)	0	0	0	0	3	-	-	-	-	-
Tremors (coarse)	0	0	0	0	1	-	-	-	-	-
4 Hour post-treatment										
Active/alert	3	3	3	1	4	3	3	2	3	4
Inactive/alert	1	1	1	3	0	1	1	2	1	0
Tremors (none)	4	4	4	4	0	4	4	4	4	4
Tremors (fine)	0	0	0	0	3	-	-	-	-	-
Tremors (coarse)	0	0	0	0	1	-	-	-	-	-
5 Hour post-treatment										
Active/alert	4	3	2	2	2	-	-	-	-	-
Inactive/alert	0	1	2	2	2	-	-	-	-	-
Tremors (none)	4	4	4	4	1	-	-	-	-	-
Tremors (fine)	0	0	0	0	3	-	-	-	-	-
6 Hour post-treatment										
Active/alert	-	-	-	-	-	3	2	2	1	2
Inactive/alert	-	-	-	-	-	1	2	2	3	2
Tremors (none)	-	-	-	-	-	4	4	4	4	3
Tremors (fine)	-	-	-	-	-	0	0	0	0	1
7 Hour post-treatment										
Active/alert	-	-	-	-	-	3	2	2	3	4

Inactive/alert	-	-	-	-	-	1	2	2	1	0
Tremors (none)	-	-	-	-	-	4	4	4	4	3
Tremors (fine)	-	-	-	-	-	0	0	0	0	1
24 Hour post-treatment										
Active/alert	4	4	2	1	2	2	3	2	3	3
Inactive/alert	0	0	2	3	2	2	1	2	1	1
Tremors (none)	4	4	4	4	4	4	4	4	4	4

Table 6.7.1/01-2 Summary of observations in phase II part one

Group (g/kg)	Females					
	0.000	**0.025**	**0.050**	**0.100**	**0.150**	**0.200**
1 Hour post-treatment						
Active/alert	2	2	2	2	2	2
Tremors (none)	2	2	2	2	2	2
2 Hour post-treatment						
Active/alert	1	2	2	2	2	1
Inactive/alert	1	0	0	0	0	0
Hyperactive	0	0	0	0	0	1
Tremors (none)	2	2	2	2	2	1
Tremors (fine)	0	0	0	0	0	1
3 Hour post-treatment						
Active/alert	2	2	2	2	2	1
Hyperactive	0	0	0	0	0	1
Tremors (none)	2	2	2	1	1	0
Tremors (fine)	0	0	0	1	1	2
4 Hour post-treatment						
Active/alert	2	1	1	2	2	0
Inactive/alert	0	1	1	0	0	1
Hyperactive	0	0	0	0	0	1
Tremors (none)	2	2	2	1	1	0
Tremors (fine)	0	0	0	1	1	1
Tremors (coarse)	0	0	0	0	0	1
5 Hour post-treatment						
Active/alert	1	1	2	2	0	0
Inactive/alert	1	1	0	0	2	1
Hyperactive	0	0	0	0	0	1
Tremors (none)	2	2	2	2	2	0
Tremors (fine)	0	0	0	0	0	2
6 Hour post-treatment						
Active/alert	2	1	2	2	1	1
Inactive/alert	0	1	0	0	1	1
Tremors (none)	2	2	2	2	2	1
Tremors (fine)	0	0	0	0	0	1
24 Hour post-treatment						
Active/alert	2	1	1	2	2	1
Inactive/alert	0	1	1	0	0	0
Hyperactive	0	0	0	0	0	1
Tremors (none)	2	2	2	2	2	2

Conclusions:

Findings from this study indicate that female rats are more sensitive to the test substance than male rats. Based upon the results of this study, dose levels of 0.04, 0.125, and 0.4g total pyrethrins/kg body weight were selected for males in the definitive acute neurotoxicity study. The doses were to be administered as a solution of pyrethrum extract in corn oil to contain 10% total pyrethrums. For females, dose levels of 0.02, 0.063, and 0.2 g total

pyrethrins/kg bodyweight were selected for use in the definitive acute neurotoxicity study. The doses were to be administered as a solution of pyrethrum extract in corn oil to contain 5% total pyrethrums. Based upon the time course of effects observed in this study, 3 to 5 hours was selected as the time for the first post-exposure evaluation in the definitive acute neurotoxicity study.

ASSESSMENT AND CONCLUSION BY APPLICANT

Assessment:

This was a GLP compliant study. The study did not follow any OECD guidelines and was used to establish dose levels and select appropriate evaluation intervals for a definitive acute oral neurotoxicity study.

Conclusion:

Appropriate dose levels and evaluations timepoints were selected for the definitive neurotoxicity study based on the findings from this study; i.e. dose levels of 0.04, 0.125, and 0.4 g total pyrethrins/kg body weight for males, and dose levels of 0.02, 0.063, and 0.2 g total pyrethrins/kg body weight for females, and 3 to 5 hours as the time for the first post-exposure evaluation time of 3 to 5 hours.

ASSESSMENT AND CONCLUSION BY RMS

RMS agrees with the assessment and the conclusion of the applicant.

The study did not follow any OECD guidelines but it is considered adequate to establish dose levels and time intervals for evaluation in a definitive acute oral neurotoxicity study.

Based on the results of the study, a dosing solution containing 10% total pyrethins and dose levels of 0.04, 0.125, and 0.4 g total pyrethrins/kg body weight were selected for males. For females in the definitive acute neurotoxicity study, a dosing solution containing 5% total pyrethins and dose levels of 0.02, 0.063, and 0.2 g total pyrethrins/kg body weight were selected. In addition, 3 to 5 hours was determined as the time for the first post-exposure evaluation time.

9) Comparative functional observational battery study of twelve commercial pyrethroid insecticides in male rats following acute oral exposure; Weiner M. L., Nemec M., Sheets L., Sargent D., & Breckenridge C., 2009; Cross reference IIA 5.7.1/03

Guidelines:

None

GLP:

Yes.

The study is acceptable as supportive information

Executive Summary:

Twelve commercial pyrethroid insecticides (technical-grade active ingredients) were evaluated individually for acute neurobehavioral manifestations of toxicity under conditions suited to assist with determining whether they act by a common mechanism of toxicity. The pyrethroids that were tested reflect a diversity of structures, including six with an α-cyano

phenoxybenzyl moiety (bcyfluthrin, l-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate and fenpropathrin) and six without this moiety (bifenthrin, S-bioallethrin, permethrin, pyrethrins, resmethrin and tefluthrin). These chemicals also present a variety of behavioural effects, including ones that are historically classified as causing a T (tremor), CS (choreoathetosis with salivation) or intermediate syndrome of intoxication, and others that have not previously been classified. Each pyrethroid that was tested consisted of the complement of isomers that occur in commercial products—a key factor for relevance for environmental and human exposure and for comparisons, since the biological activity of the individual isomers can vary tremendously.

Young-adult male Sprague–Dawley rats (10 per dose group) were administered a single dose of pyrethroid by oral gavage, in corn oil, at a volume of 5 ml/kg. Each was tested at a range of two or three dose levels, including a minimally toxic dose, to establish the more sensitive manifestations of toxicity, and a more toxic dose, to establish a more complete spectrum of neurobehavioral manifestations. Animals were evaluated using a functional observational battery (FOB) that was designed to characterize and distinguish effects classically associated with T or CS syndromes of intoxication. The FOB was performed when manifestations of toxicity were most apparent at the time of peak effect (2, 4, or 8 h post-dosing) by observers who were blinded to dose group assignment, thus avoiding possible bias. The results from this study indicate that some pyrethroids clearly exhibit the historic classification symptoms of the T and CS syndromes while others do so less obviously. Use of the statistical technique of Principal Component Analysis (PCA) further helped interpret the study findings.

These results establish manifestations of neurotoxicity in vivo that can be used as weight of evidence to determine whether pyrethroid insecticides act through a common mechanism of toxicity in mammals. Based on a review of the FOB data, analyzed by PCA, and other published data, two common mechanism groups are proposed. Group 1 (T syndrome) would include pyrethrins, bifenthrin, resmethrin, permethrin, S-bioallethrin and tefluthrin. Group 2 (CS syndrome) would include cypermethrin, deltamethrin, esfenvalerate, b-cyfluthrin and l-cyhalothrin. Fenpropathrin exhibited features of both groups.

This summary is focused on the information for pyrethrins only and will refer to the test compounds when necessary.

Material and methods:

A. MATERIALS

1. Test Material: Pyrethrins

Description: Not reported

Lot/Batch: Not reported

Purity: Not reported (Purity and stability verification for the test substances were confirmed by the respective suppliers by Certificates of Analysis or GLP analyses).

2. Vehicle: Mazola corn oil

3. Test Animals

Species: Rat

Strain: Charles River Crl:CD®(SD)IGS BR

Age: 28-29 days

Sex: Male

Weight at dosing: 219-284 g (males) and 136-182 g (females)

Source: Charles River Laboratories, Inc., Raleigh, NC

Acclimation period: At least 14-days

Diet: Certified Rodent chow® #5002 (PMI Nutrition International, Inc.) *ad libitum*

Water: Tap water *ad libitum*

Housing: three/cage by sex for the first three days of the 14-day acclimation period, and housed individually thereafter.

Environmental Conditions: Temperature: 71 ±3°F

Humidity: 50 ±20%

Photoperiod: 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

1. In-life dates Not reported

2. Animal assignment and treatment

The dose levels and the times-to-peak effect for each test substance were based on data from a rangefinding study at the same laboratory. For pyrethrins, dose levels of 400 and 800 mg/kg body weight were selected and the time to peak effect for clinical signs of toxicity was 4 hours post administration. The rats were allocated to 2 treatment groups and a control group of 10 rats/group, using a computerized randomisation procedure. At the time of group assignment, only rats with bw within ± 20% of the population mean were included. The selected route of administration was oral gavage. The vehicle and test substance formulations were administered orally by gastric intubation via a 16-gauge stainless steel gavage cannula as a single dose. The day of dose administration was termed study day 0 for that animal. Individual dosages were based on the day 0 body weight.

3. Statistics

Each mean was presented with the SD, and the number of animals (N) used to calculate the mean. The numeric data were subjected to statistical analyses by the Dunnett's Test, except for hindlimb resistance and extensor strength in the neuromuscular parameters, which were subjected to the Fisher's Exact Test. In addition, Principal Component Analysis and Factor Analysis were used for a more thorough interpretation of these data (Breckenridge *et al*., 2009).

C. METHODS

1. Observations

All animals were observed twice daily (morning and afternoon) for mortality and moribundity. Clinical observations were performed daily, except on the day of the Functional Observational Battery.

2. Body weight

Animals were randomized to groups based on body weight 1 week prior to dosing so that body weights were similar on the day of dosing. Individual body weights were recorded at randomization and prior to dose administration on study day 0. Body weights were also recorded during the FOB and prior to terminal euthanasia.

3. Functional Observational Battery (FOB):

The FOB used in this study was based on a standardized procedure developed and used by the laboratory. It is based on procedures in the US EPA OPPTS Health Effects Test Guideline 870.6200 (US EPA, 1996). The FOB was modified to include additional details (e.g., the coarseness of tremor) to distinguish findings particularly associated with pyrethroid

intoxication. Tremors were scored on a severity scale of 1–5: 1 indicates no tremors; 2 indicates slight (1.5 mm) tremors; 3 indicates moderately coarse (3 mm) tremors with slight impairment; 4 indicates markedly coarse (4.5 mm) tremors with marked impairment of locomotion and 5 indicates extremely coarse (6 mm) tremors and locomotion impossible. For aerial righting and landing footsplay, the animals landed on a well-cushioned surface and the test was not performed if the animal was judged unable to perform the test. Observations were recorded for all animals at the time of peak effect after test substance administration. Study technicians were given special training to distinguish classical symptoms of pyrethroid intoxication. Testing was performed without the technician's knowledge of dose group assignment and inter-observer reliability was established to verify consistency among the technicians by verification of training in the laboratory with standard pyrethroids.

The FOB was performed in a sound-attenuated room equipped with a white noise generator set to operate at 70 ± 10 dB, while home cage observations were performed in the animal room.

The FOB consisted of six types of observations: home cage, handling, open field, sensory, neuromuscular and physiological observations. Table below summarizes the specific parameters evaluated for each category of the FOB observations.

Type of observation	**Parameter**	
Home cage	Posture	Biting
	Convulsions/tremors	Palpebral (eyelid) closure
	Faeces consistency	
Handling	Ease of removal from cage	Ease of handling animal in hand
	Lacrimation/chromodacryorrhea	Salivation
	Pilorection	Fur appearance
	Papebral closure	Respiratory rate/character
	Eye prominence	Mucous membrane/eye/skin colour
	Red/crusty deposits	Muscle tone
Open field	Mobility	Gait
	Rearing	Arousal
	Convulsion/tremors	Urination
	Grooming	Defecation
	Bizarre/stereotypic behaviour	Gait score
	Time to first step (s)	Backing
Sensory	Approach response	Touch response
	Startle response	Tail pinch response
	Pupil response	Eyeblink response
	Forelimb response	Hindlimb response
	Air-righting response	Olfactory response
Neuromuscular	Hind limb extensor strength	Grip strength: hind and forelimb
	Hindlimb foot splay	Rotorod performance
Physiological	Body temperature	Body weight
	Catalepsy	

4. Macroscopic examination (unscheduled deaths)

Animals found dead during the study underwent a gross necropsy examination. This included, but was not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities, including viscera.

5. Schedule euthanasia:

Following clinical observations on the day after treatment, surviving animals were euthanized by carbon dioxide inhalation and discarded without necropsy.

Results and discussion:

A. OBSERVATIONS

5. Clinical signs of toxicity

Signs of toxicity in animals dosed pyrethrins had resolved in all surviving animals by the day after treatment.

6. Mortality

All animals dosed pyrethrins survived to scheduled termination.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight for test substance-treated animals was generally similar to the concurrent control group values on the day of dosing and at the scheduled euthanasia.

C. NEUROBEHAVIOURAL EVALUATIONS

3. Functional observation battery (FOB)

FOB findings at the times of peak effect for pyrethrins (Type 1-T syndrome – non-α-cyano substituent in the 3-phenoxybenzyl alcohol moiety of the molecule) are summarized in Table 6.7.1/03-1, respectively. The pyrethrins were considered to be the least potent type I pyrethroid based on FOB data.

Table 6.7.1/03-1 Summary of FOB findings for Pyrethrins

Observation (Time to peak effect 4h)	**Dose level (mg/kg)**	
	400	**800**
Home cage observations		
Sitting, head held low		
Flattened, limbs may be extended		1
Rearing		
Splayed hindlimbs		
Clonic convulsions (repetitive movement of mouth/jaw)		
Clonic convulsions (back twitches)	1	
Clonic convulsions (head/body twitches) myoclonus		1
Clonic convulsions (irregular jerking, limbs)		1
Clonic convulsions (whole body)		1
Slight tremors	1	2
Moderately coarse tremors		
Markedly coarse tremors		
Extremely coarse tremors		
Biting of self		
Handling observations		
Salivation		1 (1)
Ventral staining	1	1
Ventral wetness		
Abdominogenital wetness		
Slightly soiled fur		
Red deposits—nose		2 (1)
Pale mucous membrane		
Pale skin		
Pulsating eyes		
Exophthalmus		
Moderately difficult to remove from cage	1	
High difficulty in handling		
Open field observations		

Ataxia, excessive sway, rocks, lurches		
Slightly impaired mobility		
Moderately impaired mobility		
Walking on tiptoes	1 (1)	3 (1)
Body drags, body sways, abdomen contacts surface		
Hindlimbs splayed or dragging		
Hunched body	1	1
Gait impairment—slight		
Gait impairment—considerable		
Gait impairment—severe, cannot walk without falling		
Clonic convulsions (back twitches)		1
Clonic convulsions (head/body twitches)		2 (1)
Clonic convulsions (irregular jerking, limbs)	2	
Clonic convulsions (whole body)		
Slight tremors	1 (1)	3 (1)
Moderately coarse tremors		
Markedly coarse tremors		1
Extremely coarse tremors		
Low arousal level		
Stereotypic behavior (head flick)		
Sensory observations		
Approach reaction—no response		
Approach reaction—more energetic response (more than slight)		
Touch response—no reaction		
Touch response—more energetic response (more than slight)		
Startle response—more energetic response (more than slight)		
Olfactory orientation—no reaction		
Air-righting reflex—slightly uncoordinated		
Exaggerated hindlimb flexion	2	1
No hindlimb extension		
No forelimb extension		
Neuromuscular observations		
Reduced hindlimb resistance		

Note: Numerals represent the numbers of animals with findings. Occurrences of findings in the control group are indicated in parenthesis next to the group finding. If the finding was not observed in the control, no number has been included.

Conclusion:

Pyrethrins is a Type 1/T syndrome pyrethroid, which is consistent with its structure and other pyrethroids without an α-cyano substituent in the 3-phenoxybenzyl alcohol moiety of the molecule. Although there is no known common toxophore that mediates acute toxicity of pyrethroids, the presence of the α-cyano substituent in the 3-phenoxybenzyl alcohol moiety of the molecule confers greater potency by an estimated order of magnitude in acute lethality studies in rodents. In addition, the manifestations of the particular toxic effect of neurotoxicity can also be related to the presence or absence of the α-cyano substituent, as noted in the early distinctions of the two structural classes of the early pyrethroids (Verschoyle and Aldridge, 1980; Lawrence and Casida, 1982) and by the present study using the FOB.

In the present study the potency of the α-cyano-containing pyrethroids was generally higher than the noncyano pyrethroids. The lowest dose tested ranged from 10 to 65 mg/kg for the α-cyano pyrethroids with l-cyhalothrin (10 mg/kg) > deltamethrin and b-cyfluthrin (12.5 mg/kg) > fenpropathrin and esfenvalerate (15 mg/kg) > cypermethrin (65mg/kg). The potency of the non-cyano pyrethroids was generally lower than the α-cyano pyrethroids based on the lowest dose tested: tefluthrin (10 mg/kg) > bifenthrin (40 mg/kg) > S-

bioallethrin (150 mg/kg) > permethrin (200mg/kg) > resmethrin (350 mg/kg) > pyrethrins (400 mg/kg).

ASSESSMENT AND CONCLUSION BY APPLICANT

Assessment:

This was a GLP compliant study. This study was not evaluated for the first EU approval review of pyrethrins (DAR Vol. 3 B6, 2007). The study is not a guideline study, but the functional observations conducted are consistent with those of OECD guideline 424 (1997). The study is conducted at higher dose levels than those tested in Hermansky and Hurley (1993).

The study shows that pyrethrins are of significantly lower neurotoxicity than all other pyrethroid molecules tested. The study is conducted at higher dose levels than those tested in Hermansky and Hurley (1993) and do not therefore have any impact on the dose levels selected for human health risk assessment.

ASSESSMENT AND CONCLUSION BY RMS

RMS agrees with the assessment and the conclusion of the applicant.

According to the study, pyrethrins are considered to be the least potent type I pyrethroid (Type 1-T syndrome – non-α-cyano substituent in the 3-phenoxybenzyl alcohol moiety of the molecule) based on FOB data. Moreover, pyrethrins are of significantly lower neurotoxicity than all other pyrethroid molecules tested.

The study is conducted at higher dose levels than those tested in Hermansky and Hurley (1993) and RMS agrees that does not have any impact on the dose levels selected for human health risk assessment.

DAR - ANNEX B.9 Ecotoxicology

Active substance: Pyrethrins

*1) Pyrethrum extract (FEK-99) - acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions. Machado M.W., 1994e*

Guidelines:

In accordance with U.S.EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-3.

Testing Laboratory and dates:

[redacted]

USA conducted the study during the period December 14, 1993 to December 18, 1993.

GLP:

Yes (self-certified), with the following exceptions:

- Routine water and food contaminant screening analyses for pesticides, PCBs and metals were not collected in accordance with GLP (i.e., no distinct protocol, Study Director, etc.)
- Total organic carbon analyses for filtered seawater were not collected in accordance with GLP
- Documentation of observations made during a single interval of a preliminary exposure was recorded using pencil

These deviations were not considered to have affected the scientific validity of the study or the interpretation of the results.

The study is acceptable

Executive Summary:

In an acute toxicity laboratory study under flow-through conditions, *Mysidopsis bahia* were exposed to total Pyrethrins (57.488% purity) at nominal concentrations of 0, 0.38, 0.75, 1.5, 3.0, 6.0 µg/L over a period of 96 hours. Each concentration was testing using 20 mysid shrimp per treatment level. Dilution water and acetone solvent were also tested as negative and solvent controls, respectively.

At test termination, mean cumulative mortalities of 5%, 10% and 55% were observed among mysids exposed to the 0.34, 0.81, and 1.6 µg total Pyrethrins/L treatment levels. Sub-lethal effects were observed among several of the surviving mysids exposed to 0.81 µg total Pyrethrins/L and among all of the surviving mysid exposed to the 1.6 µg total Pyrethrins/L treatment level.

The 96-hour LC_{50} (95% confidence interval) was calculated by moving average angle analysis to be 1.4 µg total Pyrethrins/L. The 96-hour NOEC was determined to be 0.34 µg total Pyrethrins/L. Based on these results and on criteria established by Directive 67/548/EEC, pyrethrum extract (FEK-99) would be classified as very toxic to mysid shrimp.

Materials and Methods:

MATERIALS

Test Material:	Pyrethrum extract (FEK-99)
Description:	Brown liquid
Lot/Batch #:	R92-254
Purity:	57.488% total Pyrethrins
CAS #:	8003-34-7
Stability of test compound:	> 5 years at 0°C in the absence of light
Vehicle and/or positive	Acetone (solvent control)

control:	
	Dilution water (negative control)
Test organisms -	
Species:	*Mysidopsis bahia*
Source:	[redacted]
Age:	≤24 h
Acclimation conditions:	
Acclimation period:	≥14 days
Diet:	Mysid cultures were fed live brine shrimp *(Adernia salina)* nauplii twice daily
Temperature:	24°C to 26°C
Dissolved oxygen:	81% to 87%
Photoperiod:	16 h light, 8 h darkness
5. Exposure conditions:	
Temperature:	25 ± 1°C
Feed:	Mysid cultures were fed live brine shrimp *(Adernia salina)* nauplii twice daily
Photoperiod:	16 h light, 8 h darkness
Dilution Water:	
Water used in study:	Seawater piped in from the Cape Cod Canal, Bourne, Massachusetts, USA from approximately 4 meters offshore at a depth of 0.5 meters
Salinity:	31 to 32‰
pH:	7.8 to 7.9

Findings:

MORTALITY

Cumulative mortality and sublethal effect data for *Mysidopsis bahia* during 96 hours flow-through exposure to FEK-99 are given in Table B.9.1.1.

Table B.9.1.1: Cumulative mortality and sub-lethal effects for *Mysidopsis bahia* during 96 hours flow-through exposure to FEK-99

Mean measured concentration[a]	**Cumulative mortality (%)**			
	24 hour (mean)	**48 hour (mean)**	**72 hour (mean)**	**96 hour (mean)**
Control	0	0	0	0
Solvent control	0	0	0	0
0.29	0	0	0	0
0.34	0	5	5	5
0.81	5[bc]	10[bc]	10[bc]	10[fh]
1.6	5[d]	15[ef]	30[ef]	55[ef]
3.4	20[ef]	95[g]	100	100

[a]Concentrations measured as µg total Pyrethrins/L
[b]Two of the surviving mysids exhibited erratic swimming behavior
[c]One of the surviving mysids exhibited a partial loss of equilibrium.
[d]All of the surviving mysids exhibited a partial loss of equilibrium
[e]Several of the surviving mysids exhibited a complete loss of equilibrium
[f]Several of the surviving mysids exhibited a partial loss of equilibrium
[g]All of the surviving mysids exhibited a complete loss of equilibrium
[h]Several of the surviving mysids exhibited erratic swimming behavior

Conclusions:

The 96-hour LC_{50} based on mean measured concentrations of FEK-99 with *Mysidopsis bahia* was determined to be 1.4 µg/L total Pyrethrins, and the NOEC was 0.34 µg/L. Utilizing the concentration-effect response observed during this study and criteria established by Directive 67/548/EEC, pyrethrum extract (FEK 99) would be classified as very toxic to *Mysidopsis bahia*. (Machado MW 1994e).

Analytical data on concentrations in the test media

Materials and Methods:

MATERIALS
As per Machado MW 1994e, analysis by GC-ECD.

Findings:

The results of the analysis of the exposure solutions for total Pyrethrins during the in-life portion of the definitive exposure are presented in Table B.9.1.1 in the above section. Mean measured concentrations established for this study defined the exposure levels as 0.29, 0.34, 0.81, 1.6 and 3.4 µg total Pyrethrins/L. The mean measured concentrations averaged 60% of nominal (N=28) with a mean coefficient of variation of 25%. The ratio of the highest measured concentration to the lowest measured concentration at each treatment level was determined and ranged from 1.5 to 2.7.

Analyses of the QC samples resulted in measured concentrations which fell within two standard deviations of the acceptable recovery range, which exceeded the minimum acceptance criteria (i.e., within three standard deviations of the acceptable recovery range). Measured concentrations for the QC samples averaged 114% (N=5) of the nominal fortified levels (0.375 to 6.00 µg total Pyrethrins/L).

Conclusions:

Mean measured concentrations established for this study defined the exposure levels as 0.29, 0.34, 0.81, 1.6 and 3.4 µg total Pyrethrins/L. (Machado MW 1994e)

*2) Pyrethrum extract (FEK-99) - acute toxicity to eastern oyster (*Crassostrea virginica*) under flow-through conditions. Dionne E., 1994*

Guidelines:

In accordance with U.S.EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-3.

Testing Laboratory and dates:

[redacted]

USA conducted the study during the period October 22, 1993 to October 26, 1993.

GLP:

Yes (self-certified), with the following exceptions:

- Routine water and food contaminant screening analyses for pesticides, PCBs and metals were conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, Pennsylvania, USA. These data were not collected in accordance with GLP procedures (i.e. no distinct protocol, Study Director, etc.).

- Total organic carbon analyses for filtered seawater conducted were not collected in accordance with GLP procedures.

These deviations were not considered to have affected the scientific validity of the study or the interpretation of the results.

The study is acceptable according to US EPA documents. No OECD guidelines were found to be compared to this study

Executive Summary:

In an acute toxicity laboratory study under flow-through conditions, *Crassostrea virginica* were exposed to total Pyrethrins (57.488% purity) at nominal concentrations of 0, 26, 43, 72, 120 and 200 µg/L over a period of 96 hours. The Pacific oyster (*Crassostrea gigas*) and the Eastern oyster are the preferred species for the shell deposition test as they have demonstrated sensitivity to known toxicants and because a substantial database is available on each species. Eastern oysters were selected for this study based on their availability. Each concentration was tested using 40 oysters per treatment level. Dilution water and acetone solvent were also tested as negative and solvent controls, respectively.

At test termination, growth among dilution water control oysters averaged 2.6 mm. The average shell deposition observed was considered representative for this species and acceptable for establishing the relative toxicity of FEK-99 to Eastern oysters. Reduced feeding and reduced fecal and pseudofecal production were observed among oysters exposed to the 130 µg total Pyrethrins/L concentration. No sublethal effects were observed among oysters exposed to any of the remaining concentrations tested or the controls. Shell growth among oysters exposed to the control and solvent control averaged 2.6 and 2.2 mm, respectively. Shell growth among oysters exposed to 20, 45, 68 and 130 µg total Pyrethrins/L was reduced by 13, 36, 41 and 68%, respectively, and was significantly different when compared to the growth of the pooled control oysters. Shell growth reduction at the 14 µg total Pyrethrins/L treatment level was 12%.

The EC_{50} (95% confidence intervals) was calculated by linear regression to be 87 µg total Pyrethrins/L. The NOEC was determined to be 14 µg total Pyrethrins/L.

Materials and Methods:

MATERIALS	
Test Material:	Pyrethrum extract (FEK-99)
Description:	Brown liquid
Lot/Batch #:	R92-254
Purity:	57.488% total Pyrethrins
CAS #:	8003-34-7
Stability of test compound:	> 5 years at 0°C in the absence of light
Vehicle and/or positive control:	Acetone (solvent control)
	Dilution water (negative control)
Test organisms -	
Species:	*Crassostrea virginica* (Eastern oyster)
Source:	P. Cummins Oyster Co., Pasadena, Maryland, USA
Age:	Pre-spawn condition of gonadal development
Mean weight at start of the study:	Not documented
Mean standard length at start of the study:	25 to 50 mm
Acclimation conditions:	
Acclimation period:	23 days

Diet:	Supplementary algal diet of *Isochysis galbana*, clone T-Iso and *Tetraselmis maculate* besides natural presence of algae in seawater used in study.
Temperature:	19 to 21°C
Dissolved oxygen:	74 to 99% of saturation
Photoperiod:	16 hours light, 8 hours darkness
Exposure conditions:	
Temperature:	20 to 22°C
Feed:	Supplemental feedings of algae (*Isochysis galbana*) besides natural presence of algae in seawater used in study.
Photoperiod:	16 hours light, 8 hours darkness
Dilution Water:	
Water used in study:	Natural unfiltered seawater pumped from the Cape Cod Canal, Bourne, Massachusetts, USA from about 4 meters offshore at a depth of approximately 0.5 meters.
Salinity:	32‰
pH:	7.8
Water Hardness:	Not documented

Findings:

MEASURED EFFECTS

Effects of FEK-99 exposure to *Crassostrea virginica* are summarised in Table B.9.1.2.

Table B.9.1.2: Effects of FEK-99 on the shell deposition of *Crassostrea virginica* after 96 hours[a]

Mean measured concentration[b]	Mean shell deposition[c] (mm)	Mean percentage reduction[d]
Control	2.6(0.9)	NA[f]
Solvent control	2.2(0.8)	NA[f]
Pooled control	2.4(0.9)	NA[f]
14	2.1(0.7)	12
20	2.1(0.9)	13[e]
45	1.5(0.6)	36[e]
68	1.4(0.5)	41[e]
130	0.8(0.4)	68[e]

[a]The EC_{50} was calculated to be 87 µg total Pyrethrins/L. The NOEC was estimated to be 14 µg total Pyrethrins/L

[b]Concentrations expressed as µg total Pyrethrins/L

[c]The mean shell deposition is presented with the standard deviation in parentheses and represents 40 oysters/treatment

[d]The formula for the calculation of mean percent reduction is presented in the Study Protocol

[e]Significantly different as compared to the performance of the pooled control oysters

[f]NA = not applicable

OBSERVATIONS

Observations on sublethal effects were observed at test termination (96-hours), and included reduced feeding and reduced fecal and pseudofecal production among oysters exposed to the 130 µg total Pyrethrins/l concentration. No sublethal effects were observed among oysters exposed to any of the remaining concentrations tested or the controls.

Growth among dilution water control oysters at test termination averaged 2.6 mm. The growth of control organisms during this study exceeded the required minimum and was

within the historical range (0.9 - 4.5 mm). Based on these data, the average shell deposition observed during this study is considered representative for this species and acceptable for establishing the relative toxicity of FEK 99 to Eastern oysters. At test termination (96 hours), reduced feeding and reduced fecal and pseudofecal production were observed among oysters exposed to the 130 µg total Pyrethrins/L concentration. No sublethal effects were observed among oysters exposed to any of the remaining concentrations tested or the controls. Shell growth among oysters exposed to the control and solvent control averaged 2.6 and 2.2 mm, respectively. Statistical analysis determined no significant difference between shell deposition in the control and solvent control, therefore, control data were pooled for further analyses. Shell growth among oysters exposed to 20, 45, 68 and 130 µg total Pyrethrins/L was reduced by 13, 36, 41 and 68%, respectively, and was significantly different when compared to the growth of the pooled control oysters. Shell growth reduction at the 14 µg total Pyrethrins/L treatment level was 12%, which was not statistically different than the growth of the pooled control organisms. Effects observed during this test were clearly concentration-dependent.

Conclusions:

Under the conditions of this study, the 96-hour EC_{50} value for FEK 99 with *Crassostrea virginica* was determined to be 87 µg/L. The NOEC was 14 µg/L total Pyrethrins. (Dionne E 1994)

Analytical data on concentrations in the test media

Materials and Methods:

MATERIALS
As per Dionne E 1994, analysis by GC-ECD.

Findings:

The analyses of the exposure solutions for total Pyrethrins during the in-life portion of the definitive exposure period are presented in Table 9.1.3. Mean measured concentrations established for this study defined the exposure levels as 14, 20, 45, 68 and 130 µg total Pyrethrins/L. The mean measured concentrations averaged 56% of nominal (N = 20) with a mean coefficient of variation of 16%. The ratio of the highest measured concentration to the lowest measured concentration at each treatment level was determined and ranged from 1.3 to 1.5. Analyses of the Quality Control samples resulted in measured concentrations which fell within two standard deviations of the acceptable recovery range, which exceeded the minimum acceptance criteria (i.e., within three standard deviations of the acceptable recovery range). Measured concentrations for the QC samples averaged 88.8% (N = 6) of the nominal fortified levels (25.0 to 200 µg total Pyrethrins/L).

Table B.9.1.3: Mean concentrations of total Pyrethrins measured in exposure solutions during the 96-hour flow-through exposure of Eastern oysters (*Crassostrea virginica*)

Nominal concentration	**Measured concentration[ab]**		**% nominal[d]**	
	0-hour	**96-hour**	**Mean (CV)[c]**	

Control	<3.1	<3.7	NA[e]	NA
Solvent control	<3.1	<3.7	NA	NA
26	15	12	14(14)	52
43	22	19	20(17)	47
72	47	43.5	45(15)	63
120	71.5	65	68(17)	57
200	145	105	130(17)	63
Stock solution[f]	0.42	0.35	0.39	96
(25.0) QC 1[g]	27.1 (108)[h]	17.5 (70.1)		
(75.0) QC 2	79.0 (105)	55.9 (74.5)		
(200) QC 3	218 (109)	131 (65.5)		

[a]Concentrations expressed as µg total Pyrethrins/L
[b]Measured concentrations have been corrected for average QC recovery (i.e., 88.8%)
[c]CV = coefficient of variation
[d]Percent of nominal was calculated for each treatment level by dividing the mean measured concentration by the nominal concentration and multiplying by 100
[e]NA = not applicable
[f]Concentrations expressed as mg total Pyrethrins/mL
[g]QC = Quality Control sample
[h]Percent of nominal for each QC sample is presented in parentheses

Conclusions:

The geometric mean measured concentrations for the study were 14, 20, 45, 68, and 140 µg total Pyrethrins/L. (Dionne E 1994)

B8.9.1 Effects on algal growth and growth rate

1) Refined pyrethrum extract - algal growth inhibition assay. Jenkins C.A., 2003

Guidelines:

EC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC Part C, Method 3 and OECD 201

Testing Laboratory and dates:

[redacted] conducted the study during the period April 26, 2002 to November 15, 2002.

GLP:

Fully GLP compliant.

The study is acceptable.

Executive Summary:

In an algal growth inhibition assay, *Selenastrum capricornutum* cultures were exposed to water accommodated fractions of refined pyrethrum extract (57.03% total Pyrethrins) dispersed in nutrient media at nominal loading rates of 0, 0.5, 1.1, 2.42, 5.32, 11.7, 25.8 and 56.7 mg/L. The mean measured levels were 0, 0.265, 0.615, 0.889, 1.25, 1.30, 1.95 and 1.78 mg/L.

After 72 hours of exposure, neither the E_bL_{50} (median effect loading rate based on area under the growth curve) nor the E_rL_{50} (median effect loading rate based on the growth curve) values for *S. capricornutum* could be calculated because <50% inhibition occurred during the definitive test. Similar levels of inhibition were obtained at the three highest nominal loading rates, 11.7 to 56.7 mg/L. The initial measured concentrations of test

substance at these concentrations were similar (2.25 to 2.89 mg/L). Consequently, the E_bL_{50} and E_rL_{50} values were >56.7 mg/L based on the nominal loading rate and >1.95 mg/L expressed in terms of the highest mean measured level. No microscopic abnormalities were noted. The "no observed effect loading rate" (NOELR) for area under the growth curve and for growth rate was 5.32 mg/L (nominal); the NOEC was 1.25 mg/L (measured).

Materials and Methods:

MATERIALS

Test Material:	Refined pyrethrum extract
Description:	Clear amber liquid
Lot/Batch #:	FEK-99
Purity:	57.03% total Pyrethrins
CAS #:	8003-34-7
Stability of test compound:	Stability during the course of this study was demonstrated by analysis.
Vehicle and/or positive control:	Sterile culture medium
Test organisms -	
Species:	*Selenastrum capricornutum*
Strain:	CCAP 278/4
Source:	Huntingdon Research Centre culture collection of algae and protozoa, Institute of freshwater ecology, Cumbria, UK
Culture medium:	Sterile algal nutrient medium as recommended in OECD Procedure 201 and EC Directive 92/69/EEC Official Journal no L383A, part C3
Pre-culture conditions:	
Light levels:	Not documented
Photoperiod:	Continuous
Temperature:	21 to 25°C (with an occurrence of 18.1°C in the initial 24 h)
Cell density:	0.9×10^6 cells/mL, with final aliquot of secondary culture diluted to 1.0×10^4 cells/mL before use
Environmental test conditions:	
Light levels:	9800 to 8500 lux
Photoperiod:	Continuous
Temperature:	23 ± 2 °C
Incubation period:	72 hours without renewal
Cell maintenance:	Cells were maintained in volumetric flasks. Gaseous exchange and suspension of the cells were ensured by oscillating on an orbital shaker at 150 cycles/min.

Findings:

VALIDITY CRITERIA

After 72 h, the measured levels had decreased, ranging between 2 and 36% of their nominal values and 33 and 58% of their initial values. Failure to achieve the nominal concentrations at the higher levels was attributed to the limit of aqueous solubility of the test substance having been exceeded. After 72 h, a sample without algal cells showed a small decrease in the measured level compared to medium with algal cells. After 72 hours, analysis of a sample of medium containing test substance at 0.5 mg/L, which had been incubated without algal cells, showed a small decrease in the measured level compared to medium that had been incubated with algal cells (22% compared to 63%). A sample taken

at 56.7 mg/L, which had been incubated without algal cells indicated that the exposure level was maintained (97%) whereas in the presence of algal cells a loss of 42% was noted. These results indicate that the stability of the test substance was affected by the presence of algal cells.

CELL DENSITY AND GROWTH RATE

Calculated values for the levels of inhibition of growth rate and biomass are given in Table B.9.2.1. Similar levels of inhibition were observed at 11.7 to 56.7 mg/l; this was not unexpected given the similarity of the initial measured levels of the test substance at these concentrations, which approximated the limit of solubility of the test substance under the conditions of the test. The reason for the reduction in cell growth at 1.1 mg/L was unknown but could not be attributed to the presence of test substance because no adverse effects occurred at the next two highest levels.

Although less than 50% inhibition occurred at 56.7 mg/L, further testing at higher concentrations in an attempt to define an EC_{50} value was not considered necessary because the solubility of the test substance had been exceeded. Mean values of cell densities in algal cultures on each sampling occasion are presented in Table B.9.2.2.

Table B.9.2.1: Inhibition of growth of algae exposed for 72 hours to refined pyrethrum extract

Exposure concentrations (mg refined pyrethrum extract/L)	**Mean AUC[a,b] 72 h (% inhibition)**	**Mean growth rate[c] at 0-72 h (% inhibition)**	
Nominal[d]	**Measured[e]**		
Control	ND[f]	3497	7.239
0.5	0.265	3793	7.333
1.1	0.615	3038	6.892
2.42	0.889	3394	7.230
5.32	1.25	3510	7.202
11.7	1.30	3032	6.940
25.8	1.95	2792	6.854
56.7	1.78	2649	6.885

[a]AUC = area under the curve
[b] x 10^4
[c] x 10^{-2}
[d]nominal loading rates
[e]mean measured concentrations
[f]ND = none detected (<0.01 mg/L)

Table B.9.2.2: Cell density of algae exposed for 72 hours to refined pyrethrum extract

Exposure concentrations (mg refined pyrethrum extract/L)	**Mean cell densities (× 10^4 cells/mL)**			
Nominal[a]	**Measured[b]**	**24 h**	**48 h**	**72 h**
Control	ND[c]	9.91	46.2	184
0.5	0.265	12.1	50.3	196
1.1	0.615	10.6	47.0	143
2.42	0.889	8.17	44.6	182
5.32	1.25	11.6	47.8	179
11.7	1.30	9.88	45.0	148
25.8	1.95	9.20	40.0	139
56.7	1.78	7.67	34.0	142

[a]nominal loading rates
[b]mean measured concentrations
[c]ND = none detected (<0.01 mg/L)

Conclusions:

After 72 hours of exposure to Refined Pyrethrum Extract, neither the E_bL_{50}, nor the E_rL_{50} values for *Selenastrum capricornutum* Strain no (CCAP 278/4) could be calculated because less than 50% inhibition occurred during the definitive test. Similar levels of inhibition were obtained at the three highest nominal loading rates employed in the test (11.7 to 56.7 mg/L); the initial measured concentrations of test substance at these concentrations were similar (2.25 to 2.89 mg/l). Consequently, the E_bL_{50} and E_rL_{50} values were >56.7 mg/L based on the nominal loading rate and >1.95 mg/L expressed in terms of the highest mean measured level. The "no observed effect loading rate" (NOELR) for area under the growth curve and for growth rate was 5.32 mg/L (nominal); the NOEC was 1.25 mg/L (measured). (Jenkins CA 2003)

Analytical data on concentrations in the test media

Materials and Methods:

MATERIALS
As per Jenkins CA 2003 analysis by GC-FID.

Findings:

The results of chemical analysis are given in Table B.9.2.3. The overall mean measured levels of refined pyrethrum extract were 0.265 0.615, 0.889, 1.25, 1.30, 1.95 and 1.78 mg/L (lowest to highest nominal concentrations respectively). Failure to achieve the nominal concentrations at the higher levels was attributed to the limit of aqueous solubility of the test substance having been exceeded.

After 72 hours, analysis of a sample of medium containing refined pyrethrum extract at 0.5 mg/L, which had been incubated without algal cells showed a small decrease in the measured level compared to medium that had been incubated with algal cells (22% compared to 63%). These results indicate that the stability of the test substance was affected by the presence of algal cells.

Table B.9.2.3: Measured concentrations

Nominal concentration refined pyrethrum extract	**Measured refined pyrethrum extract concentrations**	**Overall geometric mean**				
	0 h	**% N**	**72 h**	**% N[b]**	**% t0[c]**	
0	ND[a]	-	ND	-	-	-
0.500	0.432	86	0.162	32	38	0.265
0.500[d]	-	-	0.339	68	78	-
1.10	0.944	86	0.401	36	42	0.615
2.42	1.48	61	0.534	22	36	0.889
5.32	1.89	35	0.823	15	44	1.25
11.7	2.25	19	0.752	6	33	1.30
25.8	2.89	11	1.31	5	45	1.95
56.7	2.34	4	1.36	2	58	1.78
56.7[d]	-	-	2.27	4	97	-

[a]ND = none detected (<0.01 mg/L)
[b]% N = measured concentration as a % of nominal concentration
[c]% t0 = measured concentration after 72 h as % of starting concentrations
[d]culture medium incubated under test condition without algal cells

Conclusions:

At the start of the test, the measured levels of refined pyrethrum extract in samples of the test cultures ranged from 86% of its nominal value at the lowest concentration (0.5 mg/L) to 4% of nominal at the highest concentration (56.7 mg/L). After 72 hours, the measured levels had decreased, ranging between 2 and 36% of their nominal values; between 33 and 58% of their initial values. The overall mean measured levels of refined pyrethrum extract were 0.265, 0.615, 0.889, 1.25, 1.30, 1.95 and 1.78 mg/L. (Jenkins CA 2003)

2) Natural Pyrethrum: algal inhibition test. Mead C., McKenzie J., 2003

Guidelines:

EC Methods for Determination of ecotoxicity annex to Directive 92/69/EEC Part C, Method 3 and OECD 201

Testing Laboratory and dates:

[redacted] conducted the study during the period April 07, 2003 to April 10, 2003.

GLP:

Fully GLP compliant.

The study is acceptable.

Executive Summary:

In an algal growth inhibition test, six replicate flasks with *Scenedesmus subspicatus* cultures were exposed to refined pyrethrum extract (76.54% total Pyrethrins) at a single nominal concentration of 2.32 mg/L for 72 hours under constant illumination and shaking at a temperature of 24 ± 1°C. Samples of the algal populations were removed daily and cell concentrations determined for each control and treatment group.

It could be concluded that EC_{50} values were greater than 2.32 mg a.s./L, and

correspondingly, the No Observed Effect Concentration was 2.32 mg a.s./L if based on nominal concentrations. EC_{50} values were greater than 1.27 mg/L and correspondingly, the NOEC was 1.27 mg/L if based on mean measured test concentrations.

Materials and Methods:

MATERIALS

Test Material:	Natural pyrethrum
Description:	Slightly cloudy, viscous liquid
Lot/Batch #:	LAB-5
Purity:	76.54% total Pyrethrins
CAS #:	Not documented
Stability of test compound:	Stability during the course of this study was demonstrated by analysis
Vehicle and/or positive control:	Dimethylformamide (DMF)
Test organisms -	
Species:	*Scenedesmus subspicatus*
Strain:	CCAP 276/20
Source:	Culture Collection of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, The Ferry House, Far Sawrey, Ambleside, Cumbria
Culture medium:	Sterile algal nutrient medium as recommended in OECD Procedure 201 and EC Directive 92/69/EEC Official Journal no L383A, part C3
Pre-culture conditions:	
Light levels:	7000 lux (approx.)
Photoperiod:	Continuous
Temperature:	21 ± 1°C
Cell density:	2.11×10^6 cells/mL
Environmental test conditions:	
Light levels:	7000 lux (approx)
Photoperiod:	Continuous
Temperature:	24 ± 1°C
Incubation period:	72 hours
Cell maintenance:	Cells were maintained in volumetric flasks. Gaseous exchange and suspension of the cells were ensured by shaking at approximately 150 rpm

Findings

VALIDITY CRITERIA

Analysis of the test preparations at 0 hours showed the measured concentrations to be 84% and 92% of the nominal value. After 72 hours, there was a marked decline in the measured concentrations to 13% and 29% of the nominal value. Stability analyses conducted indicated that the test material was stable in culture medium for the test period, and hence the decline in measured test concentrations was considered to be due to adsorption to algal cells. Recovery analyses conducted in the presence of algal cells showed that immediate adsorption to algal cells did not occur, however this does not preclude long term adsorption occurring over the test period in the presence of actively growing algal cells. Adsorption was not a factor in the stability analyses conducted, as no algal cells were present.

Given the decline in measured concentrations over the test period, the results of the test were based on the mean measured test concentrations in order to give a "worst case" analysis of the data. The mean measured test concentrations are given in Table B.9.2.4.

Table B.9.2.4: Mean measured test concentrations on replicates 1 to 6

Nominal Concentration (mg/L)	**Mean Measured Concentration (mg/L)**	**Expressed as a Percent of the Nominal Concentration (%)**
2.32 (R_1-R_3)	1.12	48
2.32 (R_4-R_6)	1.41	61
R_1-R_6 = Replicates 1 to 6		

The results were based on the mean measured test concentrations of 1.27 mg/L. The use of mean measured test concentrations in the calculation of the results of the test had no significant effect.

CELL DENSITY AND GROWTH RATE

Calculated values for the levels of inhibition of growth rate and biomass are given in Table B.9.2.5, where it is made clear that no inhibition effect was observed on both parameters. Cell densities in the test are given inTable B.9.2.6 .

The cell concentration of the control cultures increased by a factor of 108 and the cell concentration of the solvent control cultures increased by a factor of 90 during the test in line with the OECD Guideline that states the enhancement must be at least by a factor of 16 after 72 hours.

Table B.9.2.5: Inhibition of growth rate and biomass

Nominal concentration (mg/L)	**Area under curve at 72 h**	**% Inhibition**	**Growth rate (0 – 72 h)**	**% Inhibition**
Control	2.18×10^7	-	0.065	-
Solvent control	2.04×10^7	-	0.062	-
2.32	2.12×10^7	[4]	0.062	0

[4] increase in growth as compared to the controls
E_bC_{50} (72 h) >2.32 mg/L
E_rC_{50} (0 – 72 h) >2.32 mg/L

Table B.9.2.6: Cell densities of algae exposed for 72 hours to the test substance

Nominal Concentration (mg/L)		**Cell density (1) (cells per mL)**			
		0 h	**24 h**	**48 h**	**72 h**
Control	R_1	1.15×10^4	1.26×10^5	2.02×10^5	1.22×10^6
	R_2	1.01×10^4	1.26×10^5	2.11×10^5	1.21×10^6
	R_3	1.18×10^4	1.26×10^5	2.11×10^5	1.19×10^6
	Mean	1.11×10^4	1.26×10^5	2.08×10^5	1.21×10^6
Solvent control	R_1	1.23×10^4	1.24×10^5	2.25×10^5	1.01×10^6
	R_2	1.19×10^4	1.23×10^5	1.91×10^5	1.30×10^6
	R_3	1.22×10^4	1.21×10^5	2.31×10^5	9.47×10^6
	Mean	1.21×10^4	1.23×10^5	2.16×10^5	1.09×10^6
2.32	R_1	1.42×10^4	1.21×10^5	1.86×10^5	1.18×10^6
	R_2	1.43×10^4	1.22×10^5	2.03×10^5	1.19×10^6
	R_3	1.33×10^4	1.19×10^5	1.83×10^5	1.18×10^6
	R_4	1.35×10^4	1.21×10^5	2.08×10^5	1.17×10^6
	R_5	1.31×10^4	1.25×10^5	2.12×10^5	1.19×10^6
	R_6	1.32×10^4	1.22×10^5	2.10×10^5	1.21×10^6
	Mean	1.36×10^4	1.22×10^5	2.00×10^5	1.19×10^6

(1) Cell densities represent the mean number of cells per ml calculated from the mean of the cell counts from 3 counts for each of the replicate flasks
R_1 – R_6 = Replicates 1 to 6

Conclusions:

Neither the growth, nor the biomass of *Scenedesmus subspicatus* was affected by the presence of the test material over the 72-hour exposure period. The NOEC was 2.32 mg/L. Based on the mean measured test concentrations of the test media the EC_{50} values were estimated to be > 1.27 mg/L. (Mead C, McKenzie J 2003)

Analytical data on concentrations in the test media

Materials and Methods:

MATERIALS:
As per Mead C, McKenzie J 2003 analysis by HPLC with detection UV at 230nm.

Findings:

The detection system was found to have acceptable linearity (R^2 = 0.9999, ranging from 0

to 101 mg/L). The recoveries obtained (mean: 85%) allowed for a consideration of the method as being sufficiently accurate and precise for the purposes of the test. The limit of quantitation was assessed down to 0.020 mg/L and the stability and recovery from test samples prepared as in the trials showed that there was a marked decline in measured test concentrations over the test period (results given in Table B.9.2.7 to Table B.9.2.9), though this decline was considered to be due to adsorption to algal cells.

Table B.9.2.7: Verification of test concentrations

Nominal concentration (mg/L)	**Recoveries**		
	(mg/L)	**(%)**	**Mean (%)**
2.32	2.09	90	90
2.32	2.07	89	
2.32 plus algae	2.07	89	Not applicable

Table B.9.2.8: Stability results from test samples prepared as in the trials after an exposure period of 72 hours in different conditions

Nominal concentration (mg/L)	2.32
Concentration found initially (mg/L)	2.08
Concentration found after storage in light conditions (mg/L)	1.97
Expressed as a percent of the initial concentration	95
Concentration found after storage in dark conditions (mg/L)	2.20
Expressed as a percent of the initial concentration	106
Concentration found after storage in dark conditions (mg/L)-unsonicated sample	2.05
Expressed as a percent of the initial concentration	98

Table B.9.2.9: Recovery results from test samples prepared as in the trials before and after an exposure period of 72 hours

Sample	**Nominal concentration (mg/L)**	**Concentration found (mg/L)**	**Expressed as a percent of the nominal concentration (%)**
0 hours	Solvent control	<LOQ	-
	2.32 R_1-R_3	1.95	84
	2.32 R_4-R_6	2.14	92
72 hours	Solvent control	<LOQ	-
	2.32 R_1-R_3	0.294	13
	2.32 R_4-R_6	0.672	29

Conclusions:

At the start of the test, the measured levels of test item in samples of the test cultures ranged from 84% of its nominal value to 92%. After 72 hours, the measured levels had decreased, ranging between 13 and 29% of the nominal concentration. Although the addition of algae to the medium showed that they did not influence on the immediate recovery of test item, it was considered after a 72-hour exposure period, that there was a process of adsorption of the test item to the algal cells. The EC_{50} was therefore based on mean measured concentrations. (Mead C, McKenzie J 2003).

Acute fish (DAR renewal)

Data point:	KCA 8.2.1/04
Report author	Teigeler, M.
Report year	2013
Report title	Fish acute toxicity test over 96 h under flow through conditions (OECD TG 203, 1992) Acute toxicity of refined pyrethrum concentrate on the zebrafish (*Danio rerio*)
Report No	GAB-034/4-32/A
Document No	GAB-034/4-32/A
Guidelines followed in study	OECD Guideline 203 (1992) EEC method C.1 (1992)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The 96-hour acute toxicity of refined pyrethrum concentrate to the zebrafish (*Danio rerio*) was determined under flow-through conditions in a dose response test. The nominal test concentrations were 50.0, 25.0, 12.5, 6.25 and 3.125 µg/L, plus a dilution water control was tested in parallel. Seven fish were tested per test concentration and the control. The mortality and sublethal effects were determined after 3 h, 24 h, 48 h, 72 h and 96 h. The 96-hour LC_{50} was determined to be 19.8 µg refined pyrethrum concentrate/L. The NOEC based on observations of mortality and clinical signs was determined to be 13.2 µg/L (mean measured concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Refined pyrethrum concentrate
   Description: Liquid, limpid, pale yellow
   Lot/Batch: FEK-99
   Content of a.s.: Total Pyrethrins: 57.03% composed as follows: Pyrethrin 1: 37.12%, Pyrethrin 2: 19.91%
   Water solubility: emulsifiable

B. STUDY DESIGN AND METHODS

1. **Test animals:** Zebrafish (*Danio rerio*) (Teleostei, Cyprinidae)
   Total weight: Mean: 0.071 ± 0.007 g
   Total length: Mean: 2.0 + 0.3 cm
   Source: Test facility
   Acclimation: Minimum of 12 days
   Diet: Fed *ad libitum* throughout the holding period with live brine shrimp (*Artemia spp.*) *nauplii* and ground flake food Tetra Min® (Tetra Werke, Melle, Germany) once daily, except during the test as well as 24 h before test start.
2. **Dilution water:** Purified drinking water
   Total hardness: 1.1 mmol/L
   Alkalinity: 1.8 mmol/L
   pH: 7.75
   Conductivity: 261.2 µS/cm
3. **Test vessels:** Glass aquaria with a volume of 25 L
4. **Environmental conditions:**
   Temperature: 23.0 ± 2 °C
   pH: 7.9 – 8.3
   Dissolved oxygen: 77 – 114 % of oxygen saturation
   Photoperiod: 12 hours light: 12 hours darkness
5. **Animal assignment and treatment:**

Zebrafish were exposed to nominal concentrations of 50.0, 25.0, 12.5, 6.25 and 3.125 µg/L for a period of 96 hours under flow-through conditions. The test included one control with dilution water only. Seven fish each were used for the test concentrations and for the control. One test vessel per test concentration was installed.

For each replicate vessel, an individual dosage system was used. Dilution water was pumped by a water dosage pump (membrane pump, Prominent, Heidelberg, Germany) into a mixing chamber, placed on a magnetic stirrer. The stock solution was added into the mixing chamber via a stock solution dosage pump (membrane pump with a stainless-steel

head, Prominent, Heidelberg, Germany). The prepared test solution flowed into the test vessels via flexible tubes. The daily water exchange rate was 5 volumes. The dilution water control was served by dilution water only. For every test vessel a water flow rate of 5.21 L/h per vessel was adjusted, resulting in a daily turnover of 5 volumes. The flow-through system was served by test solutions at least 24 h before adding the fish. The test animals were introduced in the test vessels after the concentrations of the test substance were within an acceptable range (± 20 % of nominal concentrations). The mean fish weight resulted in a loading of 0.02 g/L test medium.

6. Dose preparation:

For the preparation of the stock solution acetone was used as a solvent. Stock solution was prepared by adding 15.7, 31.3, 62.5, 125 and 250 mg of the test substance into 20 mL of acetone. Pre-warmed brown glass bottles were used. In the pre-warmed bottles 2.2 mL of each stock solution were added, blown with nitrogen and allowed the evaporation of the solvent. The bottles were then filled with 11 L Cu-free water and allowed to stir for 24 hours. This procedure was repeated daily for each concentration.

7. Measurements and observations:

Mortality and abnormal behaviour were recorded after 3 h, 24 h, 48 h, 72 h and 96 h. Dead animals were eliminated from the vessels as soon as they are discovered.

Oxygen concentration, pH and temperature were measured directly before adding the fish and afterwards once per day.

Water samples from all test vessels of the treatment levels and the control were taken for analysis at test start, after 48 hours and at test end (after 96 hours). The active substances were analysed using LC-MS. The method validation data are presented in M-CA, Section 4, Point CA 4.1.2/27.

8. Statistics:

The test results were statistically analysed to determine LC_{10} and LC_{50} values together with 95 % confidence intervals using Probit analysis assuming log-normal distribution of the values. The computer program ToxRat was used for statistical evaluations.

II. RESULTS AND DISCUSSION

A. VALIDITY CRITERIA

- The mortality in the controls should not exceed 10% at the end of the test (observed: 0%).
- The dissolved oxygen concentration should be at least 60% of the air saturation throughout the test (observed: ≥ 77% of oxygen saturation).
- The concentration of the test substance should be at least 80% of the nominal concentration throughout the test (observed: ≥ 63.9% of nominal concentration). Therefore, the results are based on the mean measured concentrations of the test substance.

All validity criteria for OECD Guideline 203 were met for the control group.

B. MORTALITY AND SUBLETHAL EFFECTS

After three hours exposure with refined pyrethrum concentrate the fish showed already symptoms as inactivity and incoordination at mean measured concentrations of 29.8 and 42.5 µg/L. In both concentrations the fish were mainly found to be at the water surface. A mortality of 85.7% at 42.5 µg/L and 14.3 % at 29.8 µg/L occurred after 24 hours. The surviving fish still showed inactivity and incoordination at these concentrations. After 48 hours a mortality of 100 % was found at concentrations of 29.8 and 42.5 µg/L.

No clinical signs and no mortality occurred at concentrations of 3.30, 7.09 and 13.2 µg refined pyrethrum concentrate/L over the whole experimental period (Table 9.2.1-10).

Table 9.2.1-10: Effect on mortality and sub-lethal effects of zebrafish (7 fish per concentration) exposed to refined pyrethrum concentrate

Mean measured concentration (µg/L)	Cumulative mortality (%)				
	3-hours	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0	0
3.30	0	0	0	0	0
7.09	0	0	0	0	0
13.2	0	0	0	0	0
29.8	0[a,b,c]	14.3[e,f]	100	100	100
42.5	0[a,d]	85.7[g,h]	100	100	100

[a] All fish were on water surface
[b] Three fish showed incoordination
[c] One fish showed inactivity
[d] All fish showed incoordination
[e] Four surviving fish showed incoordination
[f] Six surviving fish were on water surface
[g] One surviving fish showed incoordination
[h] One surviving fish was on water surface

Based on the results of this study, the 96-hour LC_{50} was determined to be 19.8 µg refined pyrethrum concentrate/L. The NOEC, based on observation of mortality and clinical signs, was determined to be 13.2 µg/L (mean measured concentrations) (Table 9.2.1-11).

Table 9.2.1-11: LC_x values for zebrafish exposed to refined pyrethrum concentrate after 96 hours

Endpoint	Mean measured concentration (µg refined pyrethrum concentrate/L)
96 hour-LC_{10} (95% confidence intervals)	18.2 (n.d.)
96 hour-LC_{20} (95% confidence intervals)	18.7 (n.d.)
96 hour-LC_{50} (95% confidence intervals)	19.8 (n.d.)
NOEC	13.2

n.d.: not determined due to mathematical reasons or inappropriate data

C. ANALYSIS

The measured concentration of the test substance ranged from 63.9 % to 132.5 % of nominal concentration. Mean measured concentrations were calculated to be 3.30, 7.09, 13.2, 29.8 and 42.5 µg/L. The evaluation of the effects was based on the mean measured concentrations of the test substance (Table 9.2.1-12).

Table 9.2.1-12: Measured concentrations of refined pyrethrum concentrate in the exposure solutions

Nominal concentration (µg test substance/L)	Mean measured concentration (µg test substance/L)	Percent of nominal (%)
Control	n.a.	n.a.
3.125	3.30	105.6
6.25	7.09	113.4
12.5	13.2	105.8
25.0	29.8	119.2
50.0	42.5	85.0

n.a.: Not applicable

D. DEFICIENCIES

None.

III. CONCLUSION

Based on the results of this study, the 96-hour LC_{50} of refined Pyrethrum concentrate to zebrafish was determined to be 19.8 µg refined Pyrethrum concentrate/L. The NOEC, based on observation of mortality and clinical signs, was determined to be 13.2 µg/L (mean measured concentrations).

Assessment and conclusion by applicant

Assessment:

The deviation of measured test substance deviated by more than 20% from nominal. All test concentrations were analysed at the beginning and at the end of the test and at an additional point of time (48 h). The results are based on mean measured values. Concentrations refer to total pyrethrins, even though referred to as "test item" or "Pyrethrum concentrate". According to guideline OECD 203 (June 2019) fish should be observed twice per day. In the current test they were inspected twice on day 1 and once per day on days 2 -4.

Conclusion:

The study complies with the data requirements given in Commission Regulation No 283/2013.

The 96-hour LC_{50} of refined pyrethrum concentrate to zebrafish was determined to be 19.8 µg refined Pyrethrum concentrate/L. The NOEC, based on observation of mortality and clinical signs, was determined to be 13.2 µg/L (mean measured concentrations).

Assessment and conclusion by RMS

The study has been evaluated by RMS in accordance to OECD guideline 203 (1992). All validity criteria were met despite the following deviation from OECD guideline 203 (2019) : a minimum of 2 biological observations on days 2-4 should be inspected twice per day, whilst in this study biological observations were inspected only once per day during days 2-4.

Moreover, OECD guideline 203 (2019) states that « the LC_{50}, the confidence limits (95%) and the slope of the curve should be estimated using appropriate statistical methods », but in this study no confidence limits are reported due to

mathematical reasons or inappropriate data. In addition, it's reported in the KCA 8.2.1/04 (Table 14) that the variance of the slope b of probit regression is much more higher than the estimated value of b. At this regard, OECD guideline 203 (2019) specifies that « When an experiment results in only one concentration with partial mortality or no concentration with partial mortality, classical maximum likelihood methods cannot be used to estimate the LC_{50}, the slope of the concentration-response curve cannot be estimated, and a confidence interval for the LC_{50} may not be estimable. In such cases, estimates of the LC_{50} can be made using various techniques such as the Spearman-Karber method (Stephan, 1977), the binomial method (USEPA, 2002), the moving average method (ISO, 1996), or as a last resort, the graphical method (USEPA, 2002). These non classical methods can give precise LC_{50} estimates and are useful to evaluate acute fish studies yielding results that cannot be analysed using classical probit maximum likelihood techniques. » In this study, no concentrations resulted in partial mortality but linear maximum likelihood regression was used to fit the probit model. However, as response to the issue raised by zRMS, the applicant provided a statistical re-analysis of LC50 value based on binomial method (Report N. 1504359.UK0 - 6297) that confirmed the previously derived endpoint.

The recalculation of endpoints using the binomial method according to OECD 203 (2019) resulted in LC50 value of 19.83 µg/L (95% CI: 13.2 – 29.8 µg/L) for *Danio rerio* (mean measured concentrations).
The NOEC, based on observation of mortality and clinical signs, was determined to be 13.2 µg/L (mean measured concentrations).

Data point:	KCA 8.2.1/05
Report author	Teigeler, M.
Report year	2013
Report title	Fish acute toxicity test over 96 h under flow through conditions (OECD TG 203, 1992) Acute toxicity of refined pyrethrum concentrate on the three-spined stickleback (*Gasterosteus aculeatus*)
Report No	GAB-034/4-32/G
Document No	GAB-034/4-32/G
Guidelines followed in study	OECD Guideline 203 (1992) EEC method C.1 (1992)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The 96-hour acute toxicity of refined pyrethrum concentrate to the three-spined stickleback (*Gasterosteus aculeatus*) was determined under flow-through conditions in a dose response test. The nominal test concentrations were 50.0, 25.0, 12.5, 6.25 and 3.125 µg/L, plus a dilution water control was tested in parallel. Seven fish were tested per test concentration and the control. The mortality and sublethal effects were determined after 3 h, 24 h, 48 h, 72 h and 96 h. The 96-hour LC_{50} was determined to be 10.9 µg refined pyrethrum concentrate/L. The NOEC based on observations of mortality and clinical signs was determined to be 6.57 µg/L (mean measured).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Refined pyrethrum concentrate
   Description: Liquid, limpid, pale yellow
   Lot/Batch: FEK-99
   Content of a.s.: Total Pyrethrins: 57.03% composed as follows: Pyrethrin 1: 37.12%, Pyrethrin 2: 19.91%

B. STUDY DESIGN AND METHODS

1. **Test animals:** Three-spined stickleback (*Gasterosteus aculeatus*) (Teleostei, Gasterosteidae)
   Total weight: Mean: 0.66 ± 0.21 g
   Total length: Mean: 4.1 ± 0.2 cm
   Source: Collected from a pond plant at Kirchhundem-Albaum. The plant was fed with a steady stream of freshwater from a near runnel.
   Acclimation: Minimum of 12 days

	Diet:	Fed *ad libitum* throughout the holding period with live brine shrimp (*Artemia spp.*) *nauplii* and ground flake food Tetra Min® (Tetra Werke, Melle, Germany) once daily, except during the test as well as 24 h before test start.
2.	**Dilution water:**	Purified drinking water
	Total hardness:	1.1 mmol/L
	Alkalinity:	1.8 mmol/L
	pH:	7.75
Conductivity:		261.2 µS/cm
3.	**Test vessels:**	Glass aquaria with a volume of 25 L
4.	**Environmental conditions:**	
Temperature:		14.9 – 15.2 °C
pH:		7.8 – 8.4
Dissolved oxygen:		88 – 98 % of oxygen saturation
Photoperiod:		12 hours light: 12 hours darkness

5. Animal assignment and treatment:

The test fish were exposed to nominal concentrations of 50.0, 25.0, 12.5, 6.25 and 3.125 µg pyrethrins/L for a period of 96 hours under flow-through conditions. The test included one control with dilution water only. Seven fish each were used for the test concentration and for the control. One test vessel per test concentration was installed.

For each replicate vessel, an individual dosage system was used. Dilution water was pumped by a water dosage pump (membrane pump, Prominent, Heidelberg, Germany) into a mixing chamber, placed on a magnetic stirrer. The stock solution was added into the mixing chamber via a stock solution dosage pump (membrane pump with a stainless steel head, Prominent, Heidelberg, Germany). The prepared test solution flowed into the test vessels via flexible tubes. The daily water exchange rate was 5 volumes. The dilution water control was served by dilution water only. For every test vessel a water flow rate of 5.21 L/h per vessel was adjusted, resulting in a daily turnover of 5 volumes. The flow-through system was served by test solutions at least 24 h before adding the fish. The test animals were introduced in the test vessels after the concentrations of the test substance were within an acceptable range (± 20 % of nominal concentrations). The mean fish weight resulted in a loading of 0.18 g/L test medium.

6. Dose preparation:

For the preparation of the stock solution acetone was used as a solvent. Stock solution was prepared by adding 15.7, 31.3, 62.5, 125 and 250 mg of the test substance into 20 mL of acetone. Pre-warmed brown glass bottles were used. In the pre-warmed bottles 2.2 mL of each stock solution were added, blown with nitrogen and allowed the evaporation of the solvent. The bottles were then filled with 11 L Cu-free water and allowed to stir for 24 hours. This procedure was repeated daily for each concentration.

7. Measurements and observations:

Mortality and abnormal behaviour were recorded after 3 h, 24 h, 48 h, 72 h and 96 h. Dead animals were eliminated from the vessels as soon as they are discovered.

Oxygen concentration, pH and temperature were measured directly before adding the fish and afterwards once per day.

Water samples from all test vessels of the treatment levels and the control were taken for analysis at test start, after 48 hours and at test end (after 96 hours). The active substances were analysed using LC-MS. The method validation data are presented in M-CA, Section 4, Point CA 4.1.2/27.

8. Statistics:

The test results were statistically analysed to determine LC_{10} and LC_{50} values together with 95 % confidence intervals using Probit analysis assuming log-normal distribution of the values. The computer program ToxRat was used for statistical evaluations.

II. RESULTS AND DISCUSSION

A. VALIDITY CRITERIA

- The mortality in the controls should not exceed 10% at the end of the test (observed: 0%).
- The dissolved oxygen concentration should be at least 60% of the air saturation throughout the test (observed: ≥ 88% of oxygen saturation).
- The concentration of the test substance should be at least 80% of the nominal concentration throughout the test (observed: ≥ 51.8% of nominal concentration). Therefore, the results are based on the mean measured concentrations of the test substance.

All validity criteria for OECD Guideline 203 were met for the control group.

B. MORTALITY AND SUBLETHAL EFFECTS

After three hours exposure with refined pyrethrum concentrate the fish showed already symptoms as incoordination and a lateral body position at mean measured concentrations of 30.3 and 45.0 µg/L. In both concentrations the fish position was mainly on the bottom of the aquarium. A mortality of 71.4% at 45.0 µg/L, 28.6 % at 30.3 µg/L and 14.3% at 18.0 µg/L occurred after 24 hours. The surviving fish still showed incoordination and a lateral body position at these concentrations. After 48 hours a mortality of 100 % was found at a concentration of 45.0 µg/L. At concentrations of 18.0 and 30.3 µg/L the mortality was determined to be 42.9 % and 71.4 %. A 100 % mortality was found at 30.3 µg/L after 72

hours, whereas at 18.0 µg/L the mortality was 85.7 %. At the end of the test (96 hours) also the last fish in the treatment of 18.0 µg/L died and a mortality of 100 % was found.
No clinical signs and no mortality occurred at concentrations of 2.10 and 6.57 µg refined pyrethrum concentrate/L over the whole experimental period (Table 9.2.1-13).

Table 9.2.1-13: Effect on mortality and sub-lethal effects of three-spined stickleback (7 fish per test concentration) exposed to refined pyrethrum concentrate

Mean measured concentration (µg/L)	Cumulative mortality (%)				
	3-hours	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0	0
2.10	0	0	0	0	0
6.57	0	0	0	0	0
18.0	0	14.3[c]	42.9[d]	85.7[f]	100
30.3	0[a]	28.6[b]	71.4[e]	100	100
45.0	0[b]	71.4[b]	100	100	100

[a] Two surviving fish showed incoordination, a lateral body position and were mainly on the bottom of the aquarium
[b] All surviving fish showed incoordination, a lateral body position and were mainly on the bottom of the aquarium
[c] Two surviving fish showed incoordination
[d] One surviving fish showed incoordination and two surviving fish showed a lateral body position and were mainly on the bottom of the aquarium
[e] All surviving fish showed a gasping respiration, a lateral body position and were mainly on the bottom of the aquarium
[f] The surviving fish showed a lateral body position and was mainly on the bottom of the aquarium

Based on the results of this study, the 96-hour LC_{50} was determined to be 10.9 µg refined pyrethrum concentrate/L. The NOEC, based on observation of mortality and clinical signs, was determined to be 6.57 µg/L (mean measured concentrations) (Table 9.2.1-14).

Table 9.2.1-14: LC_x values for three-spined stickleback exposed to refined pyrethrum concentrate after 96 hours

Endpoint	Mean measured concentration (µg refined pyrethrum concentrate/L)
96 hour-LC_{10} (95% confidence intervals)	9.75 (n.d.)
96 hour-LC_{20} (95% confidence intervals)	10.1 (n.d.)
96 hour-LC_{50} (95% confidence intervals)	10.9 (n.d.)
NOEC	6.57

n.d.: not determined due to mathematical reasons or inappropriate data

C. ANALYSIS

The measured concentration of the test substance ranged from 51.8 % to 159.9 % of nominal concentration. Mean measured concentrations were calculated to be 2.10, 6.57, 18.0, 30.3 and 45.0 µg/L. The evaluation of the effects was based on the mean measured concentrations of the test substance (Table 9.2.1-15).

Table 9.2.1-15: Measured concentrations of refined pyrethrum concentrate in the exposure solutions

Nominal concentration (µg test substance/L)	Mean measured concentration (µg test substance/L)	Percent of nominal (%)
Control	n.a.	n.a.
3.125	2.10	67.2
6.25	6.57	105.1
12.5	18.0	144.4
25.0	30.3	121.1
50.0	45.0	90.1

n.a.: Not applicable

D. DEFICIENCIES

None.

III. CONCLUSION

Based on the results of this study, the 96-hour LC_{50} of refined pyrethrum concentrate to three-spined stickleback was determined to be 10.9 µg refined pyrethrum concentrate/L and the NOEC, based on observation of mortality and clinical signs, was determined to be 6.57 µg/L (mean measured concentrations).

ES *Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

Assessment and conclusion by applicant

Assessment:

The deviation of measured test substance deviated by more than 20% from nominal. All test concentrations were analysed at the beginning and at the end of the test and at an additional point of time (48 h). The results are based on mean measured values of total pyrethrins. According to guideline OECD 203 (June 2019) fish should be observed twice per day. In the current test they were inspected twice on day 1 and once per day on days 2 -4.

Conclusion:

The study complies with the data requirements given in Commission Regulation No 283/2013.

The 96-hour LC_{50} of refined pyrethrum concentrate to three-spined stickleback was determined to be 10.9 µg pyrethrins/L. The NOEC, based on observation of mortality and clinical signs, was determined to be 6.57 µg/L (mean measured concentrations).

Assessment and conclusion by RMS

The study has been evaluated by RMS in accordance to OECD guideline 203 (1992). All validity criteria were met despite the following deviations from OECD guideline 203 (2019) : (I) The mean total length of tested animals in the current study is 4.1 mm, in contrast with the recommended length range of 1-2 cm, (II) a minimum of 2 biological observations should be conducted on days 2-4, whilst in this study biological observations were inspected only once per day on days 2-4.

Moreover, OECD guideline 203 (2019) states that « the LC_{50}, the confidence limits (95%) and the slope of the curve should be estimated using appropriate statistical methods », but in this study no confidence limits are reported due to mathematical reasons or inappropriate data. In addition, it's reported in the KCA 8.2.1/04 (Table 14) that the variance of the slope b of probit regression is much more higher than the estimated value of b. At this regard, OECD guideline 203 (2019) specifies that « When an experiment results in only one concentration with partial mortality or no concentration with partial mortality, classical maximum likelihood methods cannot be used to estimate the LC_{50}, the slope of the concentration-response curve cannot be estimated, and a confidence interval for the LC_{50} may not be estimable. In such cases, estimates of the LC_{50} can be made using various techniques such as the Spearman-Karber method (Stephan, 1977), the binomial method (USEPA, 2002), the moving average method (ISO, 1996), or as a last resort, the graphical method (USEPA, 2002). These non classical methods can give precise LC_{50} estimates and are useful to evaluate acute fish studies yielding results that cannot be analysed using classical probit maximum likelihood techniques. » In this study, no concentrations resulted in partial mortality but linear maximum likelihood regression was used to fit the probit model. However, as response to the issue raised by zRMS, the applicant provided a statistical re-analysis of LC50 value based on binomial method (Report N. 1504359.UK0 - 6297) that confirmed the previously derived endpoint.

The recalculation of endpoints using the binomial method according to OECD 203 (2019) resulted in LC50 value of 10.88 µg/L (95% CI: 6.57 – 18.0 µg/L) for *Gasterosteus aculeatus* (mean measured concentrations).
The NOEC is 6.57 µg/L (mean measured concentrations).

Chronic fish (DAR renewal)

Data point:	KCA 8.2.2.1/02
Report author	Lee, M. R.
Report year	2012
Report title	Pyrethrins TGAI - Early Life-Stage Toxicity Test with Sheepshead Minnow (*Cyprinodon variegatus*) Following OPPTS Draft guideline 850.1400
Report No	13513.6106
Document No	N/A
Guidelines followed in study	OPPTS Draft guideline 850.1400
Deviations from current test guideline	Due to an error, one replicate in one concentration contained 25 instead of 30 organisms. In the same replicate 18 instead of 15 organisms were exposed after hatch until day 5. Days to hatch not reported.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effects of Pyrethrins TGAI to embryos and larvae of the sheepshead minnow (*Cyprinodon variegatus*) were determined under flow-through conditions during 33 days. The exposure period included a 5-day incubation period and a 28-day post hatch exposure period. The nominal test concentrations were 1.3, 2.5, 5.0, 10 and 20 µg a.s./L, plus a dilution water and a solvent (DMF) control were tested in parallel. 120 organisms in four replicates were tested per test concentration and controls. The endpoints evaluated were embryo hatching success, percentage of embryos that produce live, normal larvae at hatch, larval survival and larval growth (total leec10ngth and dry weight). The LOEC was determined to be 7.0 µg a.s./L and the NOEC was determined to be 3.5 µg a.s./L (mean measured).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Pyrethrins TGAI
   Description: Brown liquid
   Lot/Batch: 230-089
   Content of a.s.: 53.48% total Pyrethrins

B. STUDY DESIGN AND METHODS

1. **Test animals:** Sheepshead minnow (*Cyprinodon variegatus*)
   Age: Embryos of approx. 36 hours old
   Source: Aquatic Biosystems, Inc., Fort Collins, Colorado, USA
   Acclimation: The embryos were allowed to acclimate to test temperature over one hour.
   Diet: On day 5, larvae were fed once with live brine shrimp nauplii (*Artemia salina*) three times daily except during the 24 hours prior to testing.
2. **Dilution water:** Dilute, filtered natural seawater from the Cape Cod Canal, Bourne, Massachusetts.
   Salinity: 19 – 20‰
   Total organic carbon: 1.1 – 1.3 mg/L
   pH: 7.8 – 8.1
3. **Test vessels:** Glass aquaria (39 x 10 x 20 cm) with a 14.5 cm high side drain which maintained a constant volume of 6.5 L.

Embryo incubation cups were glass jars (5 cm diameter, 8 cm high) with 475-µm nylon screen bottoms.

4. Environmental conditions:

Temperature:	24 – 27°C
pH:	7.7 – 8.0
Dissolved oxygen:	49 - 99% of oxygen saturation
Salinity:	19 – 22‰
Photoperiod:	16 hours light: 8 hours darkness (830 – 1000 lux)

5. Animal assignment and treatment:

Following the acclimation period, the embryos were impartially distributed to the embryo incubation cups five a time until all cups contained 30 embryos, and they were microscopically examined for viability. The incubation cups were then suspended in the respective exposure aquaria (one cup per replicate vessel) and exposed to nominal concentrations of 1.3, 2.5, 5.0, 10 and 20 µg a.s./L during 33 days under flow-through conditions. The test included one control with dilution water only and one solvent control with DMF. There were 60 organisms per treatment level in four replicate exposure aquaria at the beginning of the test. On day 5 (completion of hatch), the surviving larvae were thinned to 515 organisms per replicate, resulting in 60 organisms per concentration. Test aquaria were impartially positioned in a water bath containing circulating water.

Prior to exposure initiation, a Harvard Apparatus syringe pump in conjunction with a 10-mL Spectrum Chromatography gas-tight syringe was calibrated to deliver 0.0097 mL/cycle of the 4.0 mg a.s./mL stock solution into the diluter system's chemical mixing chamber which also received 1.94 L/cycle of dilution water.

The exposure system consisted of an intermittent flow proportional diluter, a temperature-controlled water bath and a set of 28 exposure aquaria. Flow-splitting cells were employed to equally distribute the solutions to the replicate vessels at a rate of 250 mL of control and test solution vessel per cycle.

From exposure initiation to day 26, the diluter system was calibrated to deliver the control and test solutions to the exposure aquaria (49.5 L/aquarium/day) at a rate sufficient to provide approx. 7.6 aquarium volumes per 24-hour period, with a 90% replacement time of approx. 7 hours. From day to test termination (day 33), the diluter system was calibrated to deliver control and test solutions to the exposure aquaria (64.5 L/aquarium/day) at a rate sufficient to provide approx. 9.9 aquarium volumes per 24-hour period, with a 90% replacement time of approx. 5 hours.

During the 28-day post-hatch exposure period, biomass loading did not exceed 0.059 g/L flowing solution per day or 0.45 g/L of solution at any time, in any exposure aquarium.

6. Dose preparation:

Prior to exposure initiation and every three to four days thereafter throughout the exposure, a 4.0 mg a.s./mL stock solution was prepared by placing approx. 0.0748 g of Pyrethrins TGAI (0.0400 g as active ingredient) in a 10 mL volumetric flask and bringing it to volume with dimethylformamide (DMF). The concentration of Pyrethrins TGAI in the solution contained within the mixing chamber was equivalent to that of the highest nominal test concentration (20 µg a.s./L) and was proportionally diluted (50%) to produce the remaining nominal test concentrations (10, 5.0, 2.5 and 1.3 µg a.s./L).

Prior to exposure initiation and for the first 25 days of exposure, a 36 µL/mL solvent stock solution was prepared by diluting 36 mL of DMF to 1000 mL with purified reagent water. At exposure day 26 and for the remainder of the exposure a 120 µL/mL solvent stock solution was prepared by diluting 120 mL of DMF to 1000 mL with purified reagent water.

The concentration of DMF in the solution in the mixing chamber, as well as the high test concentration, constituted the highest DMF concentration (5.0 µg/L). A Fluid Metering, Inc. (FMI) pump was calibrated to deliver the requisite amount of the DMF stock solution par cycle to the requisite amount of dilution water per cycle which was subsequently delivered to the solvent control vessels and the treatment level solutions. For the first 25 days of exposure, an FMI pump was calibrated to deliver 0.704 mL per cycle of a 36 µL/mL DMF stock solution to 5.03 L of dilution water per cycle. From day 26 until exposure termination, an FMI pump was calibrated to deliver 0.224 mL per cycle of a 120 µL/mL DMF stock solution to 5.45 L of dilution water per cycle. Throughout the entire exposure, the DMF concentration in the solvent control and the treatment levels was 5.0 µL/mL, which was equal to the DMF concentration in the high pyrethrins test concentration

7. Measurements and observations:

Embryo mortalities were counted daily until hatching was complete (exposure day 5). The 28-day post-hatch larval exposure was initiated following completion of hatch on study day 5 and placed into their respective exposure aquaria. During the post-hatch exposure period, observations of larval survival, behaviour and appearance were made and recorded daily. Larval survival was estimated daily during the post-hatch period. At 28 days post-hatch exposure (study termination) the percent larval survival was determined. The surviving larvae were anesthetised, measured to determine the total length, dried in an oven and weighed individually to determine the dry weight.

Dissolved oxygen concentration, pH, salinity and temperature were measured daily in each aquarium at exposure initiation and in alternating replicates daily thereafter until test termination.

Water samples were removed from alternating replicate solutions of each treatment level and the controls on test days 0, 5, 11, 14, 18, 21, 25 and 33 and analysed for the concentration of pyrethrins TGAI. All exposure solution samples were analysed using gas chromatography with micron electron capture detection (GC-µECD). The method validation data are presented in M-CA, Section 4, Point CA 4.1.2/28.

8. Statistics:

Data obtained on percent embryo hatch and larval survival and growth at study termination were analysed to identify significant differences between the treatment and control organisms. Analyses were performed using the mean organism response in each aquarium group rather than individual response values. All statistical analyses were conducted at the 95% level of certainty except in the case of Shapiro-Wilks', modified Levenes' Equality of Variance and Bartlett's Tests in which the 99% level of certainty was applied. The following procedures were used:

- An equal variance t-Test was used to evaluate the endpoints and to compare the performance the dilution water control organisms with that of the solvent control organisms. For embryo hatching success and percent normal larvae at hatch, no significant difference was determined between negative control and solvent control data. For percent survival, length and dry weight at test termination, a significant difference was determined between negative control and solvent control data.
- Statistical analysis of percentage hatching success, percent normal larvae at hatch and percentage larval survival was performed following arc-sin square-root percentage transformation of data.
- The Shapiro-Wilks' Test for normality was used to compare the observed sample distribution with a normal distribution for all endpoints. All data with the exception of percent normal larvae at hatch were normally distributed when treatment data were evaluated.
- As a check on the assumption of homogeneity of variance, data for each endpoint were analysed using Bartlett's Test or modified Levenes' Equality of Variance Test. All data with the exception of percent normal larvae at hatch met the assumption of homogeneity of variance when treatment data were evaluated.
- Percent normal larvae at hatch were evaluated using Steel's Many-One-Rank Test, a non-parametric procedure to evaluate treatment effects. Embryo hatching success and larval survival at test termination data were evaluated using Dunnett's Multiple comparison Test, a parametric procedure to evaluate treatment effects.
- Length and dry weight data were evaluated using Bonferroni's Adjusted t-Test, a parametric procedure to evaluate treatment effects for these endpoints.

A computer program CETIS Version 1.8.1.1 (Ives, 2009) was used to perform the statistical computations.

II. RESULTS AND DISCUSSION

A. VALIDITY CRITERIA

- The overall survival of fertilised eggs and post-hatch success in the control and in the solvent control should be ≥ 70 and 75%, respectively (observed: ≥ 95 and 82%, respectively).
- The dissolved oxygen concentration should be > 60% of the air saturation throughout the test (observed: ≥ 49% - 99% of oxygen saturation).
- The water temperature should be within 25 ± 1.5°C between test chambers or between successive days at any time during the test (observed: 24 -27°C)
- The concentrations of the test substance were measured analytically.

The dissolved oxygen concentration was below 60% of the air saturation but it was not allowed to remain below 60% for more than 8 hours. All other validity criteria for OECD Guideline 210 were met for the control groups.

B. HATCHABILITY AND SURVIVAL

The negative control and solvent control were significantly different for larval survival, total body length and dry body weight. Therefore separate analyses were performed to assess treatment effects, i.e. first treatment groups were compared to the solvent control and then to the negative control. When comparing the treatment groups to the solvent control, the most sensitive endpoint was larval survival (LOEC = 15 µg a.s./L). When comparing the treatment groups to the dilution water control, the most sensitive endpoint was larval growth (LOEC = 7.0 µg a.s./L). Therefore treatment groups were compared against the dilution water control; data in order to determine the most conservative estimate of toxicity.

At the completion of the hatching period (day 5), embryo hatching success in the dilution water control and the solvent control averaged 95 and 98%, respectively. Embryo hatching success in in the 0.70, 1.7, 3.5, 7.0 and 15 µg a.s./L treatment levels averaged 93, 100, 96, 97 and 98% respectively, and was not statistically reduced from the dilution water control.

At the completion of the hatching period (day 5), the percent of live normal larvae in both controls averaged 100%. Percent of live normal larvae in the 0.70, 1.7, 3.5, 7.0 and 15 µg a.s./L treatment level averaged 100, 99, 100, 100 and 99% respectively, and was not statistically reduced from the dilution water control.

Following 28-days post-hatch exposure (study day 33), larval survival in the dilution water control and solvent control averaged 82 and 97%, respectively. Larval survival in the 0.70, 1.7, 3.5, 7.0 and 15 µg a.s./L treatment levels averaged 88, 93, 93, 85 and 12%, respectively. There was a significant reduction ($p < 0.05$) in larval survival among fish exposed to the 15 µg a.s./L treatment level compared to the negative control (82%). Hatchability and survival data are presented in Table 9.2.2.1-4.

Table 9.2.2.1-4: Percent embryo hatching success and normal larvae at completion of hatch (day 5) and survival of sheepshead minnow larvae following 33-day exposure (28 days post-hatch) to pyrethrins TGAI

Mean measured concentration (µg a.s./L)	Mean embryo hatching success (%)	Mean normal larvae at hatch (%)	Mean larval survival at Day 28 (%)
Control	95	100	82
Solvent control	98	100	97[a]
0.70	93	100	88
1.7	100	99	93
3.5	96	100	93
7.0	97	100	85
15	98	99	12[b]

[a] Significantly difference between the dilution water control and solvent control ($p < 0.05$)
[b] Significantly reduced compared to the dilution water control ($p < 0.05$)

C. GROWTH

At test termination, total length of larvae averaged 23.8 and 22.9 mm in the dilution water control and solvent control, respectively. Total length of larvae exposed to the 0.70, 1.7, 3.5, 7.0 and 15 µg a.s./L treatment levels averaged 23.4, 23.0, 23.0, 22.4 and 22.3 mm, respectively. Statistical analysis determined a significant reduction ($p < 0.05$) in total length among fish exposed to the 7.0 and 15 µg a.s./L treatment levels tested compared to the dilution water control (23.8 mm). The dry weight of larvae in the dilution water control and solvent control averaged 0.0536 and 0.0451 g, respectively. Dry weight of larvae exposed to the 0.70, 1.7, 3.5, 7.0 and 15 µg a.s./L treatment levels averaged 0.0501.0.0469, 0.0467, 0.0443 and 0.0457 g, respectively. Statistical analysis determined a significant reduction ($p < 0.05$) in dry weight among fish exposed to the 7.0 treatment level tested compared to the dilution water control (0.0536 g). Growth data are presented in Table 9.2.2.1-5.

Table 9.2.2.1-5: Total length and dry weight of sheepshead minnow larvae following 33-day exposure (28 days post-hatch) to pyrethrins TGAI

Mean measured concentration (µg a.s./L)	Mean total length (mm) (SD)	Mean dry weight (g) (SD)
Control	23.8 (0.64)	0.0536 (0.0048)
Solvent control	22.9 (0.32)[a]	0.0451 (0.0010)[a]
0.70	23.4 (0.48)	0.0501 (0.0039)
1.7	23.0 (0.50)	0.0469 (0.0028)
3.5	23.0 (0.24)	0.0467 (0.0012)
7.0	22.4 (0.58)[b]	0.0443 (0.0039)[b]
15	22.3 (1.55)[b]	0.0457 (0.0105)

SD: standard deviation
[a] Significantly difference between the dilution water control and solvent control ($p < 0.05$)
[b] Significantly reduced compared to the dilution water control ($p < 0.05$)

Based on the effects observed during this study, larval growth was the most sensitive indicator of toxicity, the No-Observed-Effect Concentration (NOEC) was determined to be 3.5 µg a.s./L and the Lowest-Observable-Effect Concentration (LOEC) was determined to be 7.0 µg a.s/L (mean measured).

D. ANALYSIS

The mean measured concentrations ranged from 53 to 74% of nominal with a coefficient of variation ≤10% and defined the exposure levels as 0.70, 1.7, 3.5, 7.0 and 15 µg a.s./L (Table 9.2.2.1-6).

Table 9.2.2.1-6: Measured concentrations of pyrethrins TGAI in the exposure solutions

Nominal concentration (µg a.s./L)	Mean measured concentration (µg a.s./L) (%CV)	Percent of nominal (%)
Control	n.a.	n.a.
Solvent control	n.a.	n.a.
1.3	0.70 (6.4)	53
2.5	1.7 (10)	69
5.0	3.5 (7.8)	70
10	7.0 (4.4)	70
20	15 (8.1)	74

CV: coefficient of variation
n.a.: Not applicable

D. DEFICIENCIES

Organism assignement: In the concentration of 5 µg a.s./L replicate B contained 25 eggs instead of 25. Thus 115 eggs instead of 120 eggs were distributed to the four replicates of this treatment level. Calculations of hatching success were adjusted to account for this loading error. Further 18 instead of 15 larvae were transferred to this replicate at thinning. The three additional larvae were removed on day 5.
Time to hatch was not recorded.
Food: live brine shrimp fed instead of flake food. The different food is not considered to be a deficiency.

III. CONCLUSION

Based on the results of this study, larval growth was the most sensitive indicator of the toxicity of pyrethrins TGAI to sheepshead minnow. The LOEC was determined to be 7.0 µg a.s./L and the NOEC was determined to be 3.5 µg a.s./L (mean measured).

Assessment and conclusion by applicant

Assessment:

The test was performed according to OPPTS Draft guideline 850.1400. Even though in one replicate of the 5 µg a.s./L group loading errors occurred, the study was over-all well performed and is compliant with the OECD guideline 210. It is noted that the time to hatch was not recorded. The study is suited to determine lethal concentrations and the No-Observed-Effect Concentration based on sub-lethal parameters. An EC_{10} was not calculated, as no clear dose-response was shown in the investigated parameters.

Conclusion:

Based on the results of this study, larval growth was the most sensitive indicator of the toxicity of pyrethrins TGAI to sheepshead minnow. The LOEC was determined to be 7.0 µg a.s./L and the NOEC was determined to be 3.5 µg a.s./L (mean measured).

Assessment and conclusion by RMS

The study has been evaluated by RMS in accordance to OPPTS Draft guideline 850.1400. A minor deviation from this study to the current OECD guideline regards the food for tested organisms : in this study live brine shrimp was used in contrast to the reccomended frozen brine shrimp.
As reported in OPPTS Draft guideline 850.1400 and in OECD guideline 210, for a test to be valid, the dissolved oxygen concentration must be between 60 and 100 percent of the air saturation value throughout the test, but in this study the dissolved oxygen varies from 49% - 99% of oxygen saturation. The applicant reports that the saturation was not allowed to remain below 60% for more than 8 hours. During the commenting period, the coRMS commented on this issue indicating that the drop of O2 levels could compromise test results. In order to statistically control for that, the most conservative assumption would be it negatively affected only controls or low concentrations and not higher concentrations. Thus, control values would be underestimated. To give a better estimate, the highest value of all tests should be used as control. E.g. control values would be for mean embryo hatching success 100 %, mean normal larvae at hatch 100 %, mean larval survival at day 28 97 %, mean total length (mm) (SD) 23.8 (0.64), mean dry weight (g) (SD) 0.0536 (0.0048). As for the endpoints larval growth (weight and length), the NOEC would be the same. But for the other endpoints, a change could occur. Re-calculations could only be done with the original data.

The LOEC is 7.0 µg a.s./L and the NOEC is 3.5 µg a.s./L (mean measured).

Chronic invertebrates (DAR renewal)

Data point:	KCA 8.2.5.2/01
Report author	Lee, M.R.
Report year	2013
Report title	Pyrethrum Stewardship Blend – Life Cycle Toxicity Test with Mysids (*Americamysis bahia*) Following Draft OPPTS Guideline 850.1350
Report No	13513.6105
Document No	N/A
Guidelines followed in study	Draft OPPTS Guideline 850.1350 (1996)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The chronic toxicity (full life-cycle) of Pyrethrum Stewardship Blend to the mysid, *Americamysis bahia* was determined under flow-through conditions, over 28 days. The nominal test concentrations of 0.031, 0.063, 0.13, 0.25 and 0.50 µg/L (mean measured concentrations: 0.044, 0.12, 0.25, 0.63 and 1.1 µg/L) plus a dilution water and a solvent (acetone) control were tested in parallel. Four replicates, each containing 10 Mysids, were tested per test concentration and the controls. Based on mean measured concentrations and male and female growth (total body length and dry weight, the most sensitive indicators of toxicity), the No-Observed-Effect Concentration (NOEC) was determined to be 0.25 µg/L. The Lowest-Observed-Effect Concentration (LOEC) for mysids was determined to be 0.63 µg/L. Therefore, the Maximum-

Acceptable-Toxicant Concentration (MATC) was calculated to be 0.40 µg/L. Based on linear interpolation, the 7, 14, 21 and 28-day LC_{50} values were estimated to be > 1.1 µg/L, the highest mean measured concentration tested.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	Pyrethrum Stewardship Blend
	Description:	Not specified
	Lot/Batch:	230-089
	Content of a.s.:	53.48% as total pyrethrins (29.46% as pyrethrins I and 24.02% as pyrethrins II)

B. STUDY DESIGN AND METHODS

1.	**Test animals:**	Mysid (*Americamysis bahia*)
	Age:	≤ 20 hours old at test initiation
	Source:	Smithers Viscient culture facility
	Acclimation:	not specified, in-house culture
	Diet:	Live brine shrimp (*Artemia salina*) twice daily.
2.	**Dilution water:**	Filtered natural seawater from the Cape Cod Canal, Bourne, Massachusetts
	Salinity:	20 - 21‰
	pH:	7.6 - 8.0
3.	**Test vessels:**	Glass aquarium (30 x 15 x 20 cm) with a 10-cm high side drain that maintained a constant exposure solution volume of approximately 4.5 L.
4.	**Environmental conditions:**	
	Temperature:	24 – 27 °C
	pH:	7.6 – 8.0
	Dissolved oxygen:	5.02 – 7.80 mg/L (70.3 – 105% saturation)
	Salinity:	20 - 21‰
	Photoperiod:	16 hours light: 8 hours darkness (230 - 310 lux)

5. Animal assignment and treatment:

A total of 20 mysids (5 mysids per replicate, 4 replicates per concentration and controls) were exposed to nominal test concentrations of 0.031, 0.063, 0.13, 0.25 and 0.50 µg/L, a dilution water control and a solvent (acetone) control for 28 days under flow-through conditions.

The exposure system consisted of a calibrated intermittent-flow proportional diluter (Mount and Brungs, 1967) a temperature-controlled water bath, and a set of 28 exposure aquaria. During each cycle of the diluter system, approximately 500 mL of exposure solution was delivered to each replicate test vessel with a flow-splitting accuracy of 5%. During the study, the diluter provided the exposure solutions to each test vessel at a rate of approximately 7.7 aquarium volume additions per day to provide a 90% test solution replacement rate of approximately 7 hours (Sprague, 1969).

For the first 11 days of exposure, each exposure aquarium contained two retention chambers, used to retain sexually immature mysids, constructed of glass petri dishes, 10 cm in diameter, 2 cm deep, to which a 14 cm high Nitex® screen collar (350-µm mesh size opening) was attached with silicone sealant. The solution volume within the retention chambers was approximately 785 mL. Once all mysids appeared to be sexually mature (test day 12), male and female pairs were transferred to separate pairing chambers. Following this distribution, each exposure aquarium contained one retention chamber and five pairing chambers, used to retain sexually mature male and female organisms, constructed of 6-cm diameter, 1.5 cm deep petri dishes, to which a 14 cm high Nitex® screen collar (350-µm mesh size opening) was attached with silicone sealant. Solution volume within the pairing chambers was approximately 250 mL. The study was conducted in a water bath designed to maintain the test solution temperatures at 25 ± 2 °C.

During the 28-day exposure period, biomass loading did not exceed 0.0026 g/L flowing solution per day or 0.020 g/L of solution at any time, in any exposure aquarium.

6. Dose preparation:

A primary stock solution at approximately 4.0 mg/mL was prepared by adding approximately 0.07615 g of Pyrethrum Concentrate (Stewardship Blend, 0.04073 g as active ingredient) to a 10-mL volumetric flask and bringing it to volume with acetone. Each stock solution was manually mixed by inverting the flask until the solution appeared to be homogenous. A 5.8 µL/mL solvent stock solution was prepared by diluting 5.8 mL of acetone to a final volume of 1000 mL with reagent grade water in a graduated cylinder.

Prior to exposure initiation, a Harvard Apparatus syringe pump in conjunction with a 2.5-mL Hamilton gas-tight syringe was calibrated to deliver 0.00144 mL/cycle of the 4.0 mg/mL diluter stock solution into the diluter's chemical mixing chamber which also received 1.94 L of dilution water per cycle. The mixing chamber was positioned over a magnetic stir plate and was partially submerged within an ultrasonic water bath, which aided in the solubilisation of the test substance into the dilution water. The solution contained in the mixing chamber constituted the highest target test concentration (0.50 µg/L) and was subsequently diluted (50%) to provide the remaining nominal exposure concentrations (0.25, 0.13, 0.063 and 0.031 µg/L).

The concentration of acetone in the solution in the mixing chamber and the high test concentration constituted the highest acetone concentration (0.74 µL/L). An FMI pump, in conjunction with a 500-mL graduated stock bottle, was calibrated to deliver 0.64 mL/cycle of the 5.8 µL/mL solvent stock solution to 5.0 L of dilution water per cycle which was subsequently delivered to the solvent control and treatment vessels. The acetone concentration in the solvent control and the treatment levels was 0.74 µL/L, which was equal to that of the high test concentration.

7. Measurements and observations:

Observations of stress, abnormal behaviour (including discoloration, immobilization and inability to maintain position in the water column), and survival were made at the time an F1 generation pairing chamber was established and daily thereafter for 96 hours. Dead mysids were recorded and removed from each replicate test vessel daily. Missing mysids were considered dead.

Reproductive success was calculated for each replicate aquarium (treatments and the controls) as the total number of offspring produced per female. In addition, the percentage of actively reproducing females in each replicate of each treatment and the controls was determined.

At test termination (day 28), individual lengths and weights of all surviving males and females were recorded separately for each replicate of each concentration and the controls.

Temperature, dissolved oxygen concentration, pH and salinity were measured in each replicate on day 0 and alternated between replicates daily thereafter throughout the exposure period, for each treatment level and the controls.

Water samples were removed from alternating replicate solutions of each treatment level and the control on days 0, 7, 14, 21 and 28 and analysed for pyrethrins concentration (as pyrethrins I) using liquid chromatography with mass spectrometry (LC/MS/MS). The method validation data are presented in M-CA, Section 4, Point CA 4.1.2/31.

8. Statistics:

Data obtained from the test organisms were statistically analysed to establish treatment level effects. The endpoints used for determination of significant adverse effect on F0 organisms included 28-day survival, male and female survival, growth (average dry body weight and average total body length) of both male and female mysids and reproduction (number of young released per female). All statistical conclusions were made at the 95% level of certainty except in the case of the basic assumption tests, e.g., Shapiro-Wilks' Test and Bartlett's Test, in which the 99% level of certainty was applied. The following procedures were used:

- An Equal Variance Two-Sample Test was conducted to statistically compare control to the solvent control data. For this study, no significant differences were determined between the dilution water control and solvent control, therefore the dilution water control and the solvent control were pooled to establish treatment effects for all endpoints.
- Binominal endpoints (e.g., 28-day survival, male and female survival and F1 survival) were analysed using Fisher's Exact Test with Bonferroni-Holm's Adjustment.
- The Shapiro-Wilks Test for normality was conducted and compared the observed sample distribution with a normal distribution. For this study, all continuous data met this assumption.
- As a check on the assumption of homogeneity of variance, data for each endpoint were analysed using Bartlett's Test. For this study, all continuous data met this assumption.
- All continuous endpoints met the assumptions of normal distribution and homogeneity; therefore, Dunnett's Multiple Comparison Test, a parametric procedure, was used to evaluate the data.

CETIS™ was used to perform the statistical computations.

II. RESULTS AND DISCUSSION

A. VALIDITY CRITERIA

- The F0 post-pairing survival in the negative control should be > 70% at the end of the test (observed: 79%).
- The reproductively active females in the negative control should be ≥ 75% at the end of the test (observed: 100%).
- The 10th and 90th percentile of the development stage distribution in the negative control should not differ by more than 4 stages (observed: approximately 2 stages).
- The mean number of offspring produced per female surviving in the negative control should be ≥ 3 (observed: ≥ 16.5 offspring per female)

All validity criteria of the OPPTS 850.1350 Guideline were met for the control group.

B. BIOLOGICAL RESULTS

Survival and reproductive success

The biological results have been reported based on mean measured concentrations. No behavioural abnormalities were observed during the exposure period. At test termination, mean survival of 66 and 80% was observed among male mysids in the control and solvent control, respectively (pooled control = 73%). Mean survival of 83, 88, 72, 90 and 73% was observed among male mysids exposed to the 0.044, 0.12, 0.25, 0.63 and 1.1 µg/L treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant difference in male survival among organisms exposed to any of the treatment levels tested compared to the pooled control data. At test termination, mean survival of 85 and 91% was observed among female mysids in the control and solvent control, respectively (pooled control = 88%). Mean survival of 90, 89, 85, 74 and 89% was observed among female mysids exposed to the 0.044, 0.12, 0.25, 0.63 and 1.1 µg/L treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant

ES	*Chrysanthemum cinerariaefolium,* extract from open and mature flowers of *Tanacetum cinerariifolium* obtained with hydrocarbon solvents

difference in female survival among organisms exposed to any of the treatment levels tested compared to the pooled control data.

Following 28 days of exposure, mean survival of 60 and 69% was observed among organisms in the control and solvent control, respectively (pooled control = 65%). Mean survival of 80, 67, 68, 69 and 41% was observed among mysids exposed to the 0.044, 0.12, 0.25, 0.63 and 1.1 µg/L treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined a significant difference in survival among organisms exposed to the 1.1 µg/L treatment level compared to the pooled control data.

At test termination, the mean number of offspring per female for organisms in the control and solvent control was 16.5 and 17.8, respectively (pooled control = 17.2 number offspring per female). The mean number of offspring per female was 16.3, 17.0, 13.3 and 3.1 among mysids exposed to the 0.044, 0.12, 0.25 and 0.63 µg/L treatment levels, respectively. Dunnett's Multiple Comparison Test determined a significant difference in the mean number of offspring per female among organisms exposed to 0.63 µg/L treatment level tested compared to the pooled control data. Since females exposed to the 1.1 µg/L treatment level did not produce any young, the 1.1 µg/L treatment level was excluded from statistical analysis.

Significant adult mortalities were observed at these doses; therefore young were not collected for the 1.1 µg/L treatment level or for two replicates of the 0.63 µg/L treatment level. Following the 96-hour observation period, mean percent survival of 93 and 98% was observed among F1 mysids in the control and solvent control, respectively (pooled control = 96%). Mean percent survival of 100, 98, 98 and 95% was observed among F1 mysids exposed to the 0.044, 0.12, 0.25 and 0.63 µg/L treatment levels, respectively. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant difference in F1 mysid survival among organisms exposed to any of the treatment levels statistically analysed compared to the pooled control data.

A summary of the first generation (F_0) survival, reproductive success data and the F1 generation 96-hour post-release survival is presented in Table 9.2.5.2-1.

Table 9.2.5.2-1: Mean percent first generation (F_0) survival, mean number of offspring produced per female mysids exposed to Pyrethrum Stewardship Blend during 28 days and F_1 generation 96-hour post-release survival

Mean measured concentration (µg/L)	Mean % 28-day survival (SD)	Mean no. of offspring/female (SD)	Mean % 96-hour post-release survival (SD)
Control	6 (8.9)	16.5 (5.3)	93 (9.6)
Solvent control	69 (170	17.8 (4.0)	98 (5.0)
Pooled control	65	17.2 (1.6)	96
0.044	80 (7.7)	16.3 (2.4)	100 (0)
0.12	67 (19)	17.0 (1.0)	98 (5.0)
0.25	68 (7.1)	13.3 (2.9)	98 (5.0)
0.63	69 (11)	3.1 (2.0) [b]	95 (7.1)
1.1	41 (19) [a]	0 (0) [c]	n.a. [d]

SD: Standard deviation
n.a.: Not applicable
[a] Significantly reduced compared to the pooled control based on Fisher's Exact Test with a Bonferroni-Holm Adjustment.
[b] Significantly reduced compared to the pooled control based on Dunnett's Multiple Comparison Test.
[c] Due to the survival effect observed, statistical analysis was not conducted.
[d] Data excluded from statistical analysis due to significant adult mortalities.

Growth

The average total body length of male mysids in the control and solvent control was 7.09 and 7.13 mm, respectively (pooled control = 7.11 mm). The average total body length of male mysids exposed to the 0.044, 0.12, 0.25, 0.63 and 1.1 µg/L treatment levels was 7.04, 7.05, 7.06, 6.28 and 5.85 mm, respectively. Dunnett's Multiple Comparison Test determined a significant difference in the total body length of male mysids exposed to the 0.63 and 1.1 µg/L treatment levels compared to the pooled control data.

The average total body length of female mysids in the control and solvent control was 7.29 and 7.42 mm, respectively (pooled control = 7.36 mm). The average total body length of female mysids exposed to 0.044, 0.12, 0.25, 0.63 and 1.1 µg/L treatment levels was 7.46, 7.30, 7.41, 6.77 and 6.31 mm, respectively. Dunnett's Multiple Comparison Test determined a significant difference in the total body length of female mysids exposed to the 0.63 and 1.1 µg/L treatment levels compared to the pooled control data.

The average dry body weight of male mysids in the control and solvent control was 0.81 and 0.83 mg, respectively (pooled control = 0.82 mg). The average dry body weight of male mysids exposed to the 0.044, 0.12, 0.25, 0.63 and 1.1 µg/L treatment levels was 0.82, 0.85, 0.86, 0.66 and 0.62 mg, respectively. Dunnett's Multiple Comparison Test determined a significant difference in the dry body weight of male mysids exposed to the 0.63 and 1.1 µg/L treatment levels compared to the pooled control data.

The average dry body weight of male mysids in the control and solvent control was 0.81 and 0.83 mg, respectively (pooled control = 0.82 mg). The average dry body weight of male mysids exposed to the 0.044, 0.12, 0.25, 0.63 and 1.1 µg/L treatment levels was 0.82, 0.85, 0.86, 0.66 and 0.62 mg, respectively. Dunnett's Multiple Comparison Test determined a significant difference in the dry body weight of male mysids exposed to the 0.63 and 1.1 µg/L treatment levels compared to the pooled control data. The average dry body weight of female mysids in the control and solvent control was 1.15 and 1.27 mg, respectively (pooled control = 1.21 mg). The average dry body weight of female mysids exposed to the 0.044, 0.12, 0.25, 0.63 and 1.1 µg/L treatment levels was 1.19, 1.17, 1.20, 0.87 and 0.67 mg, respectively. Dunnett's Multiple Comparison Test determined a significant difference in the dry body weight of female mysids exposed to the 0.63 and 1.1 µg/L treatment levels compared to the pooled control data.
Measurements of growth, as average total body length and average dry body weight, for all surviving adult mysids at test termination are presented in Table 9.2.5.2-2.

Table 9.2.5.2-2: Mean total body length and dry body weight of first generation (F_0) male and female mysids exposed to Pyrethrum Stewardship Blend during 28 days

Mean measured concentration (µg/L)	Mean total body length in mm (SD)		Mean dry weight in mg (SD)	
	Males	Females	Males	Females
Control	7.09 (0.28)	7.29 (0.37)	0.81 (0.11)	1.15 (0.18)
Solvent control	7.13 (0.14)	7.42 (0.10)	0.83 (0.024)	1.27 (0.072)
Pooled control	7.11 (0.07)	7.36 (0.09)	0.82 (0.03)	1.21 (0.05)
0.044	7.04 (0.13)	7.46 (0.12)	0.82 (0.046)	1.19 (0.14)
0.12	7.05 (0.049)	7.30 (0.17)	0.85 (0.030)	1.17 (0.051)
0.25	7.06 (0.21)	7.41 (0.20)	0.86 (0.063)	1.20 (0.089)
0.63	6.28 (0.15) [a]	6.77 (0.23) [a]	0.66 (0.017) [a]	0.87 (0.066) [a]
1.1	5.85 (0.30) [a]	5.31 (0.06) [a]	0.62 (0.035) [a]	0.67 (0.081) [a]

SD: Standard deviation
[a] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test.

Based on linear interpolation the 7, 14, 21 and 28-day LC_{50} values were estimated to be > 1.1 µg/L, the highest mean measured concentration tested. Based on mean measured concentrations and male and female growth (total body length and dry weight), the NOEC was determined to be 0.25 µg/L and the LOEC was determined to be 0.63 µg/L. Therefore, the MATC was calculated to be 0.40 µg/L.
Based on linear interpolation, the 7, 14, 21 and 28-day LC_{50} values were estimated to be > 1.1 µg/L, the highest mean measured concentration tested.

D. ANALYSIS

Measured concentrations of Pyrethrum Stewardship Blend were slightly variable, but maintained the expected concentration gradient (50% dilution series) throughout the exposure. The coefficient of variation for all measured concentrations ranged from 13 to 31% and defined the exposure levels as 0.044, 0.12, 0.25, 0.63 and 1.1 µg/L (Table 9.2.5.2-3).

Table 9.2.5.2-3: Measured concentrations of Pyrethrum Stewardship Blend in the exposure solutions during the 28-day life-cycle exposure of mysids

Nominal concentration (µg/L)	Mean measured concentration (µg/L) (SD)	% CV	Percent of nominal (%)
Control	n.a.	n.a.	n.a.
Solvent control	n.a.	n.a.	n.a.
0.031	0.044 (0.0096)	22	140
0.063	0.12 (0.021)	17	190
0.13	0.25 (0.033)	13	190
0.25	0.63 (0.20)	31	250
0.50	1.1 (0.25)	23	220

SD: standard deviations
CV: coefficient of variation
n.a.: Not applicable

E. DEFICIENCIES

The protocol states that during the test the diluter will be visually inspected at least twice daily and complete check of diluter functioning will be made once daily. For the entire duration of the exposure, the diluter function was completely checked twice daily. The diluter system was visually inspected and a complete check of diluter function was made twice daily to more effectively prevent malfunctions. This deviation does not have a negative impact on the results or interpretation of the study.

III. CONCLUSION

Based on mean measured concentrations and male and female growth (total body length and dry weight, the most sensitive indicators of toxicity), the NOEC of Pyrethrum Stewardship Blend to *Americamysis bahia* was determined to be 0.25 µg/L. The LOEC was determined to be 0.63 µg/L. Therefore, the MATC was calculated to be 0.40 µg/L.
Based on linear interpolation, the 7, 14, 21 and 28-day LC_{50} values were estimated to be > 1.1 µg/L, the highest mean measured Pyrethrum Stewardship Blend concentration tested.

Assessment and conclusion by applicant

Assessment:

This study was performed in 2013 according to US-requirements. The study is considered to be acceptable.

Conclusion:

The No-Observed-effect concentration (NOEC) was determined to be 0.25 µg pyrethrins/L.

Assessment and conclusion by RMS

The study has been evaluated by RMS in accordance with OPPTS Guideline 850.1350 (1996).
The following minor deviations are reported : the photoperiod of this study (16 hours light: 8 hours darkness) is not the recommended photoperiod in the OPPTS guidance (14 hours light: 10 hours darkness) ; the body length of F0 mysids should be recorded at the first observation day (depending on time of sexual maturation) and on day 28 but in this study body length was recorded only at test termination.
All validity criteria were met and the study can be considered acceptable for riskassessment.

The NOEC is 0.25 µg pyrethrins/L (mean measured concentrations).
As regards analytical methods, the expert concluded that the mothod of this study is not acceptable (method is not fully validated - results are not in accordance with RD – two different reference materials were used - materials are not compliant with RD). Moreover, the composition of batch remained uncharacterized. Please refer to Volume 4 comments.

Water/sediment organisms (DAR renewal)

B.9.2.8.1. Freshwater Amphipods

Data point:	KCA 8.2.8/01
Report author	Bradley, M.W.
Report year	2013
Report title	Pyrethrum Stewardship Blend - Acute Toxicity to Freshwater Amphipods (*Hyalella azteca*) Under Flow-Through Conditions
Report No	13513.6133
Document No	N/A
Guidelines followed in study	US EPA Draft Guideline 850.1020 (1996)
Deviations from current test guideline	None. Study not an EU data requirement.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The acute toxicity of Pyrethrum Stewardship Blend to the freshwater amphipod *Hyalella azteca* was determined under flow-through conditions for 96 hours. The nominal test concentrations were 0.25, 0.50, 1.0, 2.0 and 4.0 µg/L (mean measured concentrations: 0.10, 0.24, 0.54, 0.88 and 2.2 µg/L) plus a dilution water and a solvent (acetone) control were tested in parallel. One replicate aquarium containing three retention chambers, each with ten amphipods, was included for each test concentration and the controls.

Based on nominal concentrations, the 96-hour LC_{50} value was determined by the Trimmed Spearman-Kärber Method to be 1.5 µg/L, with 95% confidence intervals of 1.3 to 1.8 µg/L. The No-Observed-Effect Concentration (NOEC) and Lowest-Observed-Effect Concentration (LOEC) were determined to be 0.50 and 1.0 µg/L, respectively. Based on mean measured concentrations, the 96-hour LC_{50} value was by the Trimmed Spearman-Kärber Method to be 0.76 µg/L, with 95% confidence intervals of 0.64 to 0.92 µg/L. The NOEC and LOEC were determined to be 0.24 and 0.54 µg/L, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Pyrethrum Stewardship Blend
Description: Not specified
Lot/Batch: 230-089
Content of a.s.: 53.40% as total pyrethrins (29.88% as pyrethrins I and 23.52% as pyrethrins II)

B. STUDY DESIGN AND METHODS

1. **Test animals:** Freshwater amphipod (*Hyalella azteca*)
Age: 9 days old at test initiation
Source: Smithers Viscient cultures
Acclimation: 48 hours
Diet: Flaked fish food suspension (YCT) three times daily
2. **Dilution water:** Laboratory well water
Total hardness: 74 – 78 mg/L as $CaCO_3$
Total alkalinity: 28 mg/L as $CaCO_3$
pH: 6.9
Conductivity: 320 – 400 µmhos/cm
3. **Test vessels:** Glass aquaria (30 x 15 x 20 cm) with a 15-cm high side drain that maintained a constant exposure solution volume of 6.8 L.
4. **Environmental conditions:**
Temperature: 22 – 25°C
pH: 7.0 – 7.7
Dissolved oxygen: 6.4 – 8.9 mg/L
Photoperiod: 16 hours light: 8 hours darkness (220 - 490 lux)
5. **Animal assignment and treatment:**

Thirty amphipods (three chambers per treatment aquarium, each containing ten amphipods) were exposed to nominal concentrations of 0.50, 1.0, 2.0, 4.0 and 8.0 µg/L, a dilution water control and a solvent (acetone) control, in a flow-through study for a duration of 96 hours. Each exposure aquarium contained three retention chambers consisting of 250-µm mesh Nitex® screen (14 cm long) attached to a 6-cm diameter petri dish (1.5 cm high) using silicone, which remained partially submerged throughout the exposure. To initiate the study, ten amphipods were added impartially to retention chambers.

The flow-through test was conducted using an exposure system consisting of an intermittent-flow proportional diluter (Mount and Brungs, 1967), a temperature-controlled water bath and a set of seven exposure aquaria. The test system was designed to provide five concentrations of the test substance, a dilution water control and a solvent control. One replicate aquarium was included for each test concentration and the controls. The diluter delivered the control and test solutions to the exposure aquaria (68 L/aquarium/day) at a rate sufficient to provide approximately 10 aquarium volumes per 24-hour period, with a 90% replacement time of approximately 6 hours.

Test aquaria were positioned in a water bath designed to maintain the test solution's temperature 23 ± 1 °C.

6. **Dose preparation:**

A 40 µg/mL diluter stock solution was prepared prior to exposure initiation by bringing 0.01503 g of Pyrethrum Stewardship Blend (0.00803 g as active ingredient) to a volume of 200 mL with acetone. In addition, a 510 µL/mL solvent stock solution was prepared by bringing 102 mL of acetone to a volume of 200 mL with deionized water.

Prior to exposure initiation, a Harvard Apparatus syringe pump in conjunction with a 50-mL Glenco gas-tight syringe was calibrated to deliver 0.0785 mL/cycle of the 40 µg/mL diluter stock solution to the diluter's mixing chamber which also received 0.785 L of dilution water per cycle. The mixing chamber was positioned over a magnetic stir plate and was partially submerged within an ultrasonic water bath which aided in the solubilisation of the test substance into the dilution water. The solution contained in the mixing chamber constituted the highest nominal test concentration (4.0 µg/L) and was subsequently diluted (50%) to provide the remaining nominal exposure concentrations (2.0, 1.0, 0.50 and 0.25 µg/L). The concentration of acetone in the solution in the mixing chamber and the high test concentration constituted the highest acetone concentration (0.10 mL/L). A similar system was calibrated to deliver 0.0785 mL/cycle of the 510 µL/mL solvent stock solution to 0.40 L of dilution water per cycle which was subsequently delivered to the solvent control chambers. This delivery method ensured the acetone concentration in the solvent control and the treatment levels was 0.10 mL/L, which was equal to that of the high test concentration.

7. **Measurements and observations:**

The number of dead amphipods, biological observations and observations of the physical characteristics in each retention chamber was recorded at test initiation and after 24, 48, 72 and 96 hours of exposure.

The pH, dissolved oxygen concentration and temperature were measured in retention chamber A of each treatment level and the control at test initiation and in alternating retention chamber replicates daily thereafter. Continuous temperature monitoring was performed in retention chamber B of the 4.0 µg/L nominal treatment level throughout the exposure period.

One water sample was removed from each test solution and the controls for analysis of pyrethrins concentration at 0 hour (test initiation), 48 hours and 96 hours (test termination). Samples were collected from the approximate midpoint of the test vessel by pipet. All exposure solution samples were analysed for pyrethrins concentration (as pyrethrins I) using liquid chromatography with mass spectrometry (LC/MS/MS). The method validation data are presented in M-CA, Section 4, Point CA 4.1.2/32.

8. Statistics:

If at least one test concentration caused mortality of ≥ 50% of the test population, then CETIS® Version 1.8.4.20 (Ives, 2011) was used to calculate the LC_{50} values and 95% confidence intervals.

II. RESULTS AND DISCUSSION

A. VALIDITY CRITERIA

- The mortality in the controls should not exceed 10% at the end of the test (observed: 0%).
- The dissolved oxygen concentration should be between 60 and 105% of the air saturation throughout the test (observed: ≥ 75% of oxygen saturation).
- The concentration of solvent should not exceed 0.1 ml/L (observed: 0.10 mL acetone/L).
- Dissolved oxygen concentration, pH, temperature, and the concentration of test substance in test chambers should be measured at specified intervals (oberserved: Dissolved oxygen concentration, pH, temperature were measured daily and pyrethrins concentration at 0 hour, 48 hours and 96 hours).

All validity criteria for US EPA Draft Guideline OPPTS 850.1020 (1996) were met.

B. BIOLOGICAL EFFECTS

Following 96 hours of exposure, 30, 60 and 97% mortality was observed among amphipods exposed to mean measured concentrations of 0.54, 0.88 and 2.2 µg/L (1.0, 2.0 and 4.0 µg/L nominal), respectively. All surviving amphipods exposed to the 0.88 and 2.2 µg/L (2.0 and 4.0 µg/L nominal, respectively) treatment levels were observed to be lethargic. No mortality or adverse effects were observed for amphipods exposed to the remaining treatment levels tested (0.10 and 0.24 µg/L mean measured; 0.25 and 0.50 µg/L nominal) or the controls (Table 9.2.8.1-1).

Table 9.2.8.1-1: Mean cumulative mortality and biological observations of *Hyalella azteca* exposed to Pyrethrum Stewardship Blend

Mean measured concentration (µg total pyrethrins/L)	Mean cumulative mortality of organisms (%)			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
Solvent control	0	0	0	0
0.10	0	0	0	0
0.24	0	0	0	0
0.54	0	3.3	20	30
0.88	6.7[a]	30[a]	47[a]	60[a]
2.2	23[a]	47[a]	97[a]	97[a]

[a] All surviving amphipods were observed to be lethargic.

Based on nominal concentrations, the 96-hour LC_{50} value was determined by the Trimmed Spearman-Kärber Method to be 1.5 µg/L, with 95% confidence intervals of 1.3 to 1.8 µg/L. The NOEC and LOEC were determined to be 0.50 and 1.0 µg/L, respectively. Based on mean measured concentrations, the 96-hour LC_{50} value was determined by the Trimmed Spearman-Kärber Method to be 0.76 µg/L, with 95% confidence intervals of 0.64 to 0.92 µg/L. The NOEC and LOEC were determined to be 0.24 and 0.54 µg/L, respectively (Table 9.2.8.1-2).

Table 9.2.8.1-2: LC_{50}, NOEC and LOEC values for *Hyalella Azteca* exposed to Pyrethrum Stewardship Blend

Endpoint	Nominal concentration (µg total pyrethrins/L)	Mean measured concentration (µg total pyrethrins/L)
96-hour NOEC	0.50	0.24
96-hour LOEC	1.0	0.54
96-hour LC_{50} [a] (95% confidence intervals)	1.5 (1.3 – 1.8)	0.76 (0.64 – 0.92)

[a] LC_{50} values and corresponding 95% confidence intervals were determined using the Trimmed Spearman-Kärber Method

C. ANALYSIS

The mean measured concentrations ranged from 42 to 54% of nominal concentrations and defined the treatment levels tested as 0.10, 0.24, 0.54, 0.88 and 2.2 µg/L (Table 9.2.8.1-3).

Table 9.2.8.1-3: Measured concentrations of total pyrethrins in the exposure solutions

Nominal concentration (µg total pyrethrins/L)	Mean measured concentration (µg total pyrethrins/L)	Percent of nominal (%)
Control	n.a.	n.a.
Solvent control	n.a.	n.a.
0.25	0.10	42
0.50	0.24	48
1.0	0.54	54
2.0	0.88	44
4.0	2.2	54

n.a.: not applicable

D. DEFICIENCIES

During this exposure, the minimum/maximum thermometer recorded a maximum temperature of 25 °C on test day 3 whereas the protocol stated that the test temperature would be maintained at 23 ± 1 °C. Since this temperature is within the tolerance of the test species, this deviation did not have a negative impact on the results or interpretation of the study.

III. CONCLUSION

Based on nominal concentrations, the 96-hour LC_{50} value for Pyrethrum Stewardship Blend and *Hyalella azteca* was determined by the Trimmed Spearman-Kärber Method to be 1.5 µg/L, with 95% confidence intervals of 1.3 to 1.8 µg/L. The No-Observed-Effect Concentration (NOEC) and Lowest-Observed-Effect Concentration (LOEC) were determined to be 0.50 and 1.0 µg/L, respectively. Based on mean measured concentrations, the 96-hour LC_{50} value was determined by the Trimmed Spearman-Kärber Method to be 0.76 µg/L, with 95% confidence intervals of 0.64 to 0.92 µg/L. The NOEC and LOEC were determined to be 0.24 and 0.54 µg/L, respectively.

Assessment and conclusion by applicant

Assessment:

The study was performed according to US EPA Draft Guideline 850.1020 (1996) and is not a data requirement for EU registration

Conclusion:

The 96-hour LC_{50} value was determined to be 0.76 µg/L. The NOEC and LOEC were determined to be 0.24 and 0.54 µg/L, respectively.

Assessment and conclusion by RMS

The study was performed in accordance with US EPA Draft Guideline 850.1020 (1996) and respected the validity criteria.
The following minor deviation is highlighted : in this study the temperature was maintained between 22 – 25°C and the EPA guideline recommends to maintain a temperature of 18 ± 1 °C. However, many other studies show that *Hyalella azteca* can be successfully reared at higher temperature and then this deviation does not invalidate the study.

The 96-hour LC_{50} is 0.76 (95% confidence limits : 0.64 to 0.92 µg/L) based on mean measured concentration.
The NOEC and LOEC is 0.24 and 0.54 µg/L, respectively, based on mean measured concentration.

As regards analytical methods, the expert concluded that the mothod of this study is not acceptable (method is not fully validated - results are not in accordance with RD – two different reference materials were used - materials are not compliant with RD). Moreover, the composition of batch remained uncharacterized and the study is not accepted by method experts. Please refer to Volume 4 comments.