Committee for Risk Assessment

RAC

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

Adopted

8 June 2023

8 June 2023

CLH-O-0000007334-76-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: ***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**

The proposal was submitted by **Spain** and received by RAC on **1 March 2022.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Spain has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **25 April 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **24 June 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Laure Geoffroy**

Co-Rapporteur, appointed by RAC: **Veda Varnai**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 June 2023** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	H332 H302 H317 H400 H410	GHS07 GHS09 Wng	H332 H302 H317 H410		inhalation: ATE = 2.5 mg/L (dusts and mists) oral: ATE = 700 mg/kg bw M=100 M=10	
RAC opinion	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 STOT SE 1 (nervous system) STOT RE 2 (respiratory system, inhalation route) Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H332 H302 H370 H373 H317 H400 H410	GHS07 GHS08 Dgr	H332 H302 H370 H373 H317 H410		inhalation ATE = 2.6 mg/L (dusts and mists) oral: ATE = 730 mg/kg bw M=1000 M=100	
Resulting Annex VI entry if agreed by COM	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 STOT SE 1 (nervous system) STOT RE 2 (respiratory system, inhalation route) Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H332 H302 H370 H373 H317 H400 H410	GHS07 GHS08 Dgr	H332 H302 H370 H373 H317 H410		inhalation: ATE = 2.6 mg/L (dusts and mists) oral: ATE = 730 mg/kg bw M=1000 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

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-oxocyclopent-2-enyl (E)-(1R)-trans -3-(2-methoxycarbonylprop-1-enyl)-2,2 dimethylcyclopropane carboxylate	204-454-2 121-20-0	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)
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[1].

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)[2] 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 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,

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1 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)

2 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).

the Applicant stated that the technical equivalence assessment studies supported read-across between the two extraction methods.

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**.

HUMAN HEALTH HAZARD EVALUATION

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[3] 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).

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

[3] **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.

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[4] 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.**

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.

[4] **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.

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.

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[5] (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' 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[6]. 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

5 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).

6 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).

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 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[7], 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 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".

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7 "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."

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.

Human data on respiratory tract effects of pyrethrins

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)[8].

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 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.

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8 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).

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[9] 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 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.

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.

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[9] 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.

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[10] 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 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.

[10] 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.

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.

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[11] 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 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

[11] 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.

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.

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.

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.

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.

Liver changes

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.

Neurotoxicity and repeated-dose studies

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.

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[12]. 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)

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

[12] 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.

(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).

Bacterial gene mutation assays

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)[13]. 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 activation, no mutagenic effect (i.e. no increase in revertant colony numbers as compared to the vehicle control)

[13] Pyrethrins I group include pyrethrin 1, cinerin 1, and jasmolin 1, and Pyrethrins II group consists of pyrethrin 2, cinerin 2 and jasmolin 2.

was observed (Mutation Factor (MF)[14] 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.

In vitro unscheduled DNA synthesis assay in rat primary hepatocytes (BRA, MGK and SCJ, 1989)

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.

In vitro mammalian chromosomal aberration test in Chinese hamster ovary (CHO-K_1) cells (KPIC/BRA, MGK and SCJ, 1996)

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) 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).

[14] Mutation Factor: mean number of revertants on the test item plate / mean number of revertants on the vehicle control plate

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)[15].

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 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

[15] 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

- 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.

In vitro cytogenicity studies in mammalian cells (MN) (KPIC, 2016a; MGK, 2016b; BRA, 2016c)

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**.

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*.

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").

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[16]: 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. 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[17], 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

[16] 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%).

[17] *CAR = constitutive androstane receptor; PXR = pregnane X receptor*

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);

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[18], 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

[18] 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.

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.

Thyroid follicular cell adenomas relevance

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. 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.

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.

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[19], 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], 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

[19] RAC notes that for a lot of females, body weight measurements are missing during the period of gestation/lactation.

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.

<u>Dams</u>

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.

<u>Offspring</u>
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[20]; 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

[20] 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."

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).

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.

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[21]. 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**.

[21] 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.

ENVIRONMENTAL HAZARD EVALUATION

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

<u>Acute Aquatic Toxicity</u>

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 Reliability 1	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 % jasmolin 2 Lot/Batch: XX-82-J2 Purity: 96.6 % 0.02 mg/L for each analyte	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.	**Key study**	Perboni, A. (2015) IUCLID 10.1.1.1.a (BRA, MGK, SCJ and KPIC)
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. (2002) IIIA-7.1.1.2.1 (MGK, BRA and 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 III 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:	Water/sediment pond	^{14}C-pyrethrin 1 (Batch number CFQ 7390; Purity	**Mineralisation 4 % after 30 days**	Key study only one sediment was used for	Robinson, R. A.; Wisocky, M.J. (1994), Doc III

Environmental Fate GLP Reliability 2		98.7 % - 99.7 %) ca. 10 ppm	**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 minor degradates. were detected at various intervals, none exceeding 5 % of the initial concentration of pyrethrin 1 at any point	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.	A7.1.2.2.2 (KPIC) (MGK, BRA and SCJ)
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 concen-trations 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)

Guideline	Species	Endpoint	Test material	Design	Duration	LC/ EC_{50}	Remarks	Reference
OECD TG 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) EC_{50} 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	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)								
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.**

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.**

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ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Annex 3 Records of the targeted consultation following the submission of additional information on specific target organ toxicity (STOT SE and STOT RE)

Appendix

Summary table of oral and dermal **short-term studies**

Study, Reference, Method	**Findings**	**GV for:** **STOT RE 1** **STOT RE 2** **Effects ≤ GV?**
14-day dietary toxicity study in mice (KPIC, 1987) GLP; guideline not stated, range-finding study Test item: FEK-99 blend; purity: 57.57% Doses: 0, 5000 and 7000 ppm, → 0, 858 and 1185 mg/kg bw/d total pyrethrins 50 CD-1 males/group Note from RAR: the study is considered fit for purpose, pending on conclusion on storage stability issues	**858 mg/kg bw/d**: Food consumption: 5% ↓ week 1, with no effect on body wt Liver wt: ↑ 49% absolute, 46% relative wt **1185 mg/kg bw/d**: Mortality: one animal died on study day 2 Food consumption: up to 6↓ week 1 and 2, with no effect on body wt Liver wt: ↑ 56% absolute, 53% relative wt (haematology, clinical chemistry, and macro or micro histopath. not performed) **NOAEL**: not determined **LOAEL**: 858 mg/kg bw/d total pyrethrins (↑ liver wt)	≤ 64 mg/kg bw/d ≤ 643 mg/kg bw/d **No**
14-day dietary toxicity study in rats (KPIC, year not stated) Non-GLP, non-guideline Test item: Pyrethrum Extract 2 (Lot No. FE K87, FNB 86-2-36A; purity: 54.6%) Doses: 0, 940, 2810, 5640, and 9400 ppm → 0, 72, 216, 434, and 700 mg/kg bw/d total pyrethrins 10 males/group	**72 – 434 mg/kg bw/d**: no treatment-related toxicity **700 mg/kg bw/d**: ↓ 19% bw gain (haematology, clinical chemistry, and macro or micro histopath. not reported) No mortality and no dose related clinical signs **NOAEL**: 434 mg/kg bw/d total pyrethrins **LOAEL**: 700 mg/kg bw/d total pyrethrins (↓ bw gain)	≤ 64 mg/kg bw/d ≤ 643 mg/kg bw/d **No**
21-day dermal study in rabbits (KPIC/BRA, MGK and SCJ, 1992) GLP; OECD 410 Test item: FEK-99; purity: 57.57% Doses: 0, 100, 300, and 1000 mg/kg bw/d Pyrethrum extract → 0, 57.6, 172, and 575 mg/kg bw/d total pyrethrins, 5 days/week New Zealand White rabbits, 5/sex/group	No treatment-related local or systemic toxicity **NOAEL**: 575 mg/kg bw/d total pyrethrins **LOAEL**: not determined	≤ 86 mg/kg bw/d ≤ 857 mg/kg bw/d **No**

Summary table of oral and inhalation **sub-chronic studies**

Study, Reference, Method	**Findings**	**GV for:** **STOT RE 1** **STOT RE 2** **Effects ≤GV?**
90-day dietary dose range finding study in mice (KPIC/MGK, BRA, and SCJ, 1988a) GLP; OECD TG 408 but only in the frame of range-finding study (no haemotology and biochemistry); deviations: on day 8 male control mice accidentally received the 1000 ppm dose Test item: Pyrethrum Extract (batch 011831-00, FEK-99; purity 57.574%) Doses: 0, 300, 1000, 3000, 10000, 30000 ppm actual pyrethrins → 0, 49, 167, 490, and 1667 mg/kg bw/d total pyrethrins in males and 0, 58, 208, 594, and 1876 mg/kg bw/d total pyrethrins in females 15 CD-1 mice/sex/dose	**49/58 mg/kg bw/d**: Liver absolute wt: ↑ 12% in males Liver relative wt: ↑ 13% in males **167/208 mg/kg bw/d**: Liver absolute wt: ↑ 14% in males Liver relative wt: ↑ 16% in males **490/594 mg/kg bw/d**: Liver absolute wt: ↑ 42% in males, 33% in females Liver relative wt: ↑ 38% in males, 28% in females Histopathology: hepatocellular hypertrophy (2/10 males, 3/10 females, vs. 0 in controls) **1667/1876 mg/kg bw/d**: Mortality: 4/15 males, 2/15 females Clinical signs: neurotoxicity Liver wt: >70% ↑ in abs. and rel. wt in both sexes Histopathology: hepatocellular hypertrophy (10/10 males and females); liver congestion (10/15 males) **30000 ppm**: Mortality: 100% Clinical signs: neurotoxicity At doses <30000 ppm no effects on body weight **NOAEL**: 167 and 208 mg/kg bw/d in males and females **LOAEL**: 490 and 594 mg/kg bw/d in males and females (liver hypertrophy)	≤ 10 mg/kg bw/d ≤ 100 mg/kg bw/d **Yes**
90-day dietary dose range finding study in rats (KPIC/MGK, BRA, and SCJ, 1988b) GLP; OECD TG 408 Test item: Pyrethrum Extract (batch 011831-00, FEK-99; purity 57.574%) Doses: 0, 300, 1000, 3000, 10000, 20000 ppm actual pyrethrins → 0, 18, 59, 177, 612, and 1238 mg/kg/d in males and 0, 23, 77, 229, 742, and 1500 mg/kg/d in females 15 CD rats/sex/group	**18/23** and **59/77 mg/kg bw/d**: no treatment-related toxicity **177/229 mg/kg bw/d**: Haematology: Hb ↓ 7% females Clinical chemistry: AST ↑ 2-fold males; ALT ↑ 5-fold males (non-significantly) Liver absolute wt: ↑ 13% males, 26% females Liver relative wt: ↑ 23% males, 29% females Kidney relative wt: ↑ 16% males, 9% females (no effect on absolute wt) **612/742 mg/kg bw/d**: Mortality: 1/15 females Clinical signs: neurotoxicity Body wt: ↓ 11% males, 16% females;	≤ 10 mg/kg bw/d ≤ 100 mg/kg bw/d **No**

	Food consumption: ↓ 5% males, 16 % females Haematology: E ↓ 8% females; Hb ↓ 11% females Clinical chemistry: AST ↑ 50% males; ALT ↑ 5-fold males; urea nitrogen ↑ 30% males, 29% females Liver absolute wt: ↑ 42% males, 62% females Liver relative wt: ↑ 63% males, 87% females Kidney relative wt: ↑ 36% males, 19% females (no effect on absolute wt) Liver congestion **1238/1500 mg/kg bw/d**: Mortality: 1/15 males, 12/15 females Clinical signs: neurotoxicity Body wt: ↓ 22% males, 15% females; Food consumption: ↓ 15% males and females Haematology: E ↓ 9% females; Hb ↓ 10% males, 20% females Clinical chemistry: urea nitrogen ↑ 36% males, 29% females; creatinine ↑ 50% females Liver absolute wt: ↑ 55% males, 2-fold females Liver relative wt: ↑ >2-fold, males and females Kidney relative wt: ↑ 37% males, 18% females (no effect on absolute wt) Liver congestion Histopathology: no clear treatment-related findings at any dose level **NOAEL**: 59 and 77 mg/kg bw/d in males and females **LOAEL**: 177 and 229 mg/kg bw/d in males and females (↑ liver and kidney wt; ↑ AST and ALT in males)	
8-week dietary dose range finding study in dogs (KPIC/MGK, BRA, and SCJ, 1988c) GLP; comparable to OECD TG 409 Test item: Pyrethrum Extract (batch 011831-00, FEK-99; purity 57.574%) Doses: 0, 600, 1000, 3000, 6000 ppm actual pyrethrins → 0, 19, 31, 90, and 177 mg/kg bw/d total pyrethrins in males, 0, 20, 30, 98, and 208 mg/kg bw/d total pyrethrins in females 2 Beagle dogs/sex/dose	**19/20 mg/kg bw/d**: no treatment-related effects **31/30 mg/kg bw/d**: Liver absolute wt: ↑ 15% in females Testes weight: ↓ 13% **90/98 mg/kg bw/d**: Clinical signs: inappetence, thin appearance, ataxia, trembling, oily coat, impaired limb function, shallow breathing Haematology: E ↓ 11%, Hb ↓ 10% males Liver absolute wt: ↑ 25% females Testes wt: ↓ 24% **177/208 mg/kg bw/d**: Mortality: 1/2 males, 2/2 females	≤ 16 mg/kg bw/d ≤ 166 mg/kg bw/d **Yes**

	Body wt: ↓ 32% males, 39% females Food consumption: ↓ 29% males, 16% females Clinical signs: neurotoxicity Haematology: E ↓ 25% males, Hb ↓ 26% males Clinical chemistry (males): ↑ 2-fold in AST, ALT, and creatine phosphokinase; cholesterol ↓ 36%, glucose ↓ 22%, urea nitrogen ↑ 46% Liver absolute wt: ↑ 28% in males Kidney absolute wt: ↑ 14% in males Testes wt: ↓ 27% Histopathology: no clear treatment-related findings at any dose level **NOAEL**: 19 and 20 mg/kg bw/d total pyrethrins in males and females **LOAEL**: 31 and 30 mg/kg bw/d total pyrethrins in males and females (↑ liver and ↓ testes wt)	
90-day inhalation toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1992) GLP; OECD TG 413 (deviations: temperature and humidity partially fluctuated in a wider range) Test item: Pyrethrum extract (FEK-99; purity 57.574%) Dose: analytical conc. of 0, 11, 30, 100 and 356 mg/m^3, i.e. 0.01, 0.03, 0.1, 0.36 mg/L, approximately corresponding to 0, 3, 8, 28, and 100 mg/kg bw/day total pyrethrins Whole body exposure, 6 hours/day, 5 days/week MMAD = 2.7±1.7 µm 15 CD rats/sex/group	**0.01 mg/L**: Histopathology: ↑ in incidence and severity of changes in upper respiratory tract **0.03 mg/L**: Clinical signs: secretory symptoms (nasal discharge, dried material in the facial area) Histopathology: ↑ in incidence and severity of changes in upper respiratory tract **0.10 mg/L**: Clinical signs: secretory symptoms, tremors Body wt: ↓ 5% males and females (week 14) Body wt gain: ↓ 9% in males, 13% in females (week 14) Food consumption: ↓ 9% in males (week 1, but no ↓ in week 14) Histopathology: ↑ in incidence and severity of changes in upper respiratory tract **0.36 mg/L**: Mortality: 1/15 males* Clinical signs: secretory symptoms, neurotoxicity Body wt: ↓ 4% in males, 8% in females (week 14) Body wt gain: ↓ 9% in males, 17% in females (week 14) Food consumption week 1: ↓ 9% in males, 5% in females (week 1, no ↓ in week 14 in either sex) Haematology: leukocytes ↑ 32% in females	≤ 0.2 mg/L/6h/d ≤ 1 mg/L/6h/d **Yes**

	Liver absolute wt: ↑ 11% in males, 19% in females Liver relative wt: ↑ 17% in males, 33% in females Histopathology: ↑ in incidence and severity of changes in upper respiratory tract Histopathological changes in larynx, nasoturbinates, nasopharynx and lungs are considered to be localised responses, indicative of respiratory irritation (presented in separate tables in "Additional key elements" in the section on STOT SE). **$NOAEL_{systemic\ effects}$**: 0.03 mg/L **$LOAEL_{systemic\ effects}$**: 0.10 mg/L (neurological clinical signs, ↓ bw gain) **$NOAEL_{local\ effects}$**: not determined **$LOAEL_{local\ effects}$**: 0.01 mg/L (histopathological changes in the upper respiratory tract) *Two animals died in this group, but one death was considered accidental - misalignment of the aerosol generator allowed test substance to drop on the animal.	
1-year dietary study in dogs (KPIC/MGK, BRA, and SCJ, 1990) GLP; comparable to OECD TG 452 Test item: Pyrethrum Extract (batch 011831-00, FEK-99; purity 57.574%) Doses: 0, 100, 500, and 2500 ppm actual pyrethrins → 0, 2.7, 14, and 69 mg/kg bw/d total pyrethrins in males and 0, 2.9, 15, and 78 mg/kg bw/d total pyrethrins in females 4 Beagle dogs/sex/group	**2.7/2.9** and **14/15 mg/kg bw/d**: no treatment-related effects **69/78 mg/kg bw/d**: Body wt gain: ↓ 30% in females Haematology: leukocytes ↑ 2-fold; segmented neutrophils ↑ 2.7-fold in females; E ↓ 13%, Hb ↓ 14%, Hct ↓ 12% in males Clinical chemistry: ALT ↑ 30-40% in females (but at the high end of the normal range) Liver absolute wt: ↑ 30% in males, 25% in females Liver relative wt: ↑ 28% in males, 30% in females Histopathology: no treatment-related findings at any dose level **NOAEL**: 14 and 15 mg/kg bw/d total pyrethrins in males and females **LOAEL**: 69 and 78 mg/kg bw/d total pyrethrins in males and females (body wt gain, liver and haematological changes)	≤ 2.5 mg/kg bw/d ≤ 25 mg/kg bw/d **No**

wt = weight; E = erythrocytes; Hb = haemoglobin; Hct = haematocrit; ALT = alanine transaminase; AST = aspartate aminotransferase

Summary table of oral **long-term studies**

Study, Reference, Method	**Findings**	**GV for:** **STOT RE 1** **STOT RE 2** **Effects ≤GV?**
2-year dietary toxicity study in rats (KPIC/MGK, BRA, and SCJ, 1990a) GLP; OECD TG 453; deviations: haematological examination and urinalysis not performed at 3 months; peripheral nerve microscopic examination not conducted, however sciatic nerve examined instead; 2 control groups instead of 1 Test item: Pyrethrum Extract (FEK-99; purity: 57.574%) Doses: 0, 100, 1000 and 3000 ppm Pyrethrum Extract → 0, 4.6, 45 and 136 mg total pyrethrins/kg bw/d in males and 0, 5.6, 58, and 180 mg total pyrethrins/kg bw/d in females 60 CD rats/sex/dose, for 104 weeks	**4.6/5.6 mg/kg bw/d**: no treatment-related effects **45/58 mg/kg bw/d**: Pathology: Liver - accentuated lobulation ↑ 2.5-fold in males Thyroid - hyperplasia and follicular adenoma ↑ in males (8% vs. 0-3% in controls) **136/180 mg/kg bw/d**: Body weight: ↓ 6% in males and 11% in females (week 78; no difference at week 104) Clinical chemistry: ALT ↑ 7-fold and AST ↑ 29-fold in males Pathology: Liver - accentuated lobulation ↑ 2-fold in males; adenomas ↑ in females (8% vs. 0-2% in controls) Thyroid: hyperplasia ↑ in males and females (12% and 8% vs. 0-3% in controls); follicular adenomas ↑ in males and females (8% vs. 0-3% in controls); **NOAEL**: 4.6 and 5.6 mg/kg bw/d in males and females **LOAEL**: 45 and 58 mg/kg bw/d in males and females (liver and thyroid changes)	≤ 1.2 mg/kg bw/d ≤ 12 mg/kg bw/d **No**
18-month dietary oncogenicity study in mice (KPIC/MGK, BRA, and SCJ, 1990b) GLP; OECD TG 453, with some deviations (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) Test item: Pyrethrum Extract (FEK-99 blend; purity: 57.574%) Doses: 0, 100, 2500 and 5000 ppm actual pyrethrins → 0, 14, 361 and 715 mg total pyrethrins/kg bw/day in males and 0, 17, 430 and 869 mg pyrethrins/kg bw/d in females 60 CD-1 mice/sex/group for 18 months	**14/17 mg/kg bw/d**: no treatment-related effects **361/430 mg/kg bw/d**: Liver absolute wt: ↑ 25% and 23% in males and females Liver relative wt: ↑ 26% and 20% in males and females Pathology: Liver - discoloured dark in females (12% vs. 0 in controls); vacuolar fatty change in males (13% vs. 1.7% in controls) **715/869 mg/kg bw/d**: Mortality: 2/60; otherwise survival similar to controls Clinical signs: neurotoxicity, only during 1st week of study Liver absolute wt: ↑ 34% and 33% in males and females Liver relative wt: ↑ 37% and 35% in males and females Pathology: Liver - discoloured dark in males and females (33% and 25%, respectively, vs. 0 – 1.7% in controls): vacuolar fatty	≤ 1.7 mg/kg bw/d ≤ 17 mg/kg bw/d **No**

	change in males (23% vs. 1.7% in controls) **NOAEL**: 14 and 17 mg/kg bw/d in males and females **LOAEL**: 361 and 430 mg/kg bw/d in males and females (liver changes)	

Summary table of **reproductive toxicity study**

Study, Reference, Method	**Findings**	**GV for:** **STOT RE 1** **STOT RE 2** **Effects ≤GV?**
Range finding study for rat teratology study (KPIC/MGK, BRA, and SCJ, 1987) GLP, non-guideline Test item: Pyrethrum Extract (FEK-99; purity 57.574%) Doses: 0, 37.5, 75, 150, 300 and 600 mg actual Pyrethrins/kg bw/day → 0, 39, 78, 156, 313, and 625 mg total pyrethrins/kg bw/day; 6-15 gestation day (GD) CD rats, 5 groups of 5 mated females each	**Dams** **39 mg/kg bw/d**: no treatment-related effects **78 mg/kg bw/d**: tremors **156 - 6250 mg/kg bw/d**: mortality, convulsions and/or tremors **Foetuses** **39 and 78 mg/kg bw/d**: no treatment-related effects **$NOAEL_{maternal}$**: 39 mg/kg bw/d **$LOAEL_{maternal}$**: 78 mg/kg bw/d (neurological signs) **$NOAEL_{foetal}$**: 78 mg/kg bw/d	≤ 90 mg/kg bw/d ≤ 900 mg/kg bw/d **Yes**
Range finding study for rabbit teratology study (KPIC/MGK, BRA, and SCJ, 1987b-range finding) GLP, non-guideline Test item: Pyrethrum Extract (Blend FEK-99; purity 57.574%) Doses: 0, 39, 78, 156, 313, and 625 mg total pyrethrins/kg bw/day; 7-19 GD 5 New Zealand White rabbits/group	**Does** **39-156 mg/kg bw/day**: no treatment-related effects **313 mg/kg bw/day**: weight loss, tremors **625 mg/kg bw/day**: mortality, tremors/convulsions, weight loss **Foetuses** **625 mg/kg bw/day**: high postimplantation loss **$NOAEL_{maternal}$**: 156 mg/kg bw/d **$LOAEL_{maternal}$**: 313 mg/kg bw/d (neurological signs, weight loss) **$NOAEL_{foetal}$**: 78 mg/kg bw/d **$LOAEL_{foetal}$**: 313 mg/kg bw/d	≤ 69 mg/kg bw/d ≤ 692 mg/kg bw/d **Yes**
Definitive rabbit teratology study (KPIC/MGK, BRA, and SCJ, 1987b) GLP, OECD TG 414 414 with some deviations (number of females lower than recommended; animals dosed on GD 7-19; food consumption not recorded; maternal body weight measured less frequently than recommended) Test item: Pyrethrum Extract (Blend FEK-99; purity 57.574%) Doses: 0, 26, 104, and 260 mg total pyrethrins/kg bw/day; 7-19 GD	**Does** **26 mg/kg bw/day**: no treatment-related effects **104 mg/kg bw/day**: excessive salivation and arched head post-dose on GD 19; ↓ 36% bw gain during treatment period, but no difference *vs.* control at the end of gestation **260 mg/kg bw/day**: excessive salivation, arched head and/or laboured breathing observed post-dose on GD 18 or 19; body weight loss (38 g, i.e. 1%) during treatment period, but no difference *vs.* control at the end of gestation **Foetuses** **260 mg/kg bw/day**: 1/16 totally resorbed litter; 1/16 doe aborted near	≤ 69 mg/kg bw/d ≤ 692 mg/kg bw/d **Yes**

16 New Zealand White rabbits/group	term - unclear if these findings are treatment-related **$NOAEL_{maternal}$**: 26 mg/kg bw/d **$LOAEL_{maternal}$**: 104 mg/kg bw/d (neurological signs, reduced bw gain) **$NOAEL_{foetal}$**: 104 mg/kg bw/d **$LOAEL_{foetal}$**: 260 mg/kg bw (resorbed litter; abortion; unclear if treatment-related)	