Committee for Risk Assessment

RAC

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

Isopropyl (2*E*,4*E*,7*S*)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate; S-methoprene

EC Number: -
CAS Number: 65733-16-6

CLH-O-0000001412-86-114/F

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

Adopted

3 June 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: S-Methoprene

EC Number: Not available

CAS Number: 65733-16-6

Index Number: Not available

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Version number: 1 **Date:** July 2015

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPROPYL (2E,4E,7S)-11-METHOXY-3,7,11-TRIMETHYLDODECA-2,4-DIENOATE; S-METHOPRENE

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPROPYL (2E,4E,7S)-11-METHOXY-3,7,11-TRIMETHYLDODECA-2,4-DIENOATE; S-METHOPRENE

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	S-Methoprene
EC number:	Not available
CAS number:	65733-16-6
Annex VI Index number:	Not available
Degree of purity:	Min. 95% w/w
Impurities:	Confidential Information. See Confidential Data & Information, S-Methoprene CAR. (See Technical dossier in IUCLID 5, section 1.2)

1.2 Harmonised classification and labelling proposal

S-Methoprene is not classified according to CLP Regulation (EC) No. 1272/2008.

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	No current entry
Current proposal for consideration by RAC	Aquatic Acute 1 H400 Aquatic Chronic 1 H410 Acute M-factor of 1 and Chronic M-factor of 1
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1 H400 Aquatic Chronic 1 H410 Acute M-factor of 1 and Chronic M-factor of 1

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON ISOPROPYL (2E,4E,7S)-11-METHOXY-3,7,11-TRIMETHYLDODECA-2,4-DIENOATE; S-METHOPRENE

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	-	-	-	Conclusive but not sufficient for classification
2.2.	Flammable gases	-	-	-	Not applicable to liquids
2.3.	Flammable aerosols	-	-	-	Not applicable to liquids
2.4.	Oxidizing gases	-	-	-	Not applicable to liquids
2.5.	Gases under pressure	-	-	-	Not applicable to liquids
2.6.	Flammable liquids	-	-	-	Conclusive but not sufficient for classification
2.7.	Flammable solids	-	-	-	Not applicable to liquids
2.8.	Self-reactive substances and mixtures	-	-	-	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	-	-	-	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	-	-	-	Not applicable to liquids
2.11.	Self-heating substances and mixtures	-	-	-	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	-	-	Conclusive but not sufficient for classification
2.13.	Oxidizing liquids	-	-	-	Conclusive but not sufficient for classification
2.14.	Oxidizing solids	-	-	-	Not applicable to liquids
2.15.	Organic peroxides	-	-	-	Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	-	-	-	Data lacking
3.1.	Acute toxicity - oral	-	-	-	-

	Acute toxicity - dermal	-	-	-	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	-	-	-	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	-	-	-	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	-	-	-	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	-	-	-	Data lacking
3.4.	Skin sensitisation	-	-	-	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	-	-	-	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	-	-	-	-
3.7.	Reproductive toxicity	-	-	-	-
3.8.	Specific target organ toxicity –single exposure	-	-	-	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	-	-	-	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	-	-	-	Data lacking
4.1.	Hazardous to the aquatic environment	H400 H410	Acute M-factor.=.1 Chronic M-factor = 1	none	
5.1.	Hazardous to the ozone layer	-	-	-	

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Pictogram  GHS09

Signal word: Warning

Hazard statements: H410 Very toxic to aquatic life with long lasting effects.

Precautionary statements: P273 Avoid release to the environment

P391 Collect spillage

P501 Dispose of contents/ container in accordance with applicable regulations

Acute M-factor of 1 and **Chronic M-factor of 1**

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

S-Methoprene has no previous human health or environmental classification and labeling elements.

2.2 Short summary of the scientific justification for the CLH proposal

Physical Effects CLH proposal

No physical effects classification proposal is required for S-Methoprene.

Health Effects CLH proposal

No health effects classification proposal is required for S-Methoprene.

Environmental CLH proposal

- H400 (which is implicit in the H410 labelling) follows from the acute toxicity of the active substance to *Daphnia magna*: LC_{50} < 1 mg a.s./L (48 hour LC_{50} = 0.22 mg a.s./L). An M-factor of 1 is applicable based on 0.1 < LC_{50} ≤1 mg a.s./l.
- H410 follows from the chronic toxicity of the active substance to *Daphnia magna*: NOEC ≤ 1 mg a.s./L (NOEC = 0.019 mg/L) and the fact that the active substance is not rapidly degradable. Additionally, the logKow > 6. An M-factor of 1 is applicable based on 0.01 < NOEC ≤ 0.1 mg/l.
- GHS09 Pictogram is required for ‘Aquatic acute 1’ and ‘Aquatic chronic 1’ category substance.
- Signal word ‘Warning’ is required for ‘Aquatic acute 1’ and ‘Aquatic chronic 1’ category substance.
- The statements P273, P391 and P501 follow a general precautionary approach for dangerous substances.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

There is no current harmonised classification and labelling for S-Methoprene in Annex VI, Table 3.1 under the CLP Regulation.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

There is no current harmonised classification and labelling for S-Methoprene in Annex VI, Table 3.1 under the CLP Regulation.

2.4 Current self-classification and labelling

Not available - no information regarding S-Methoprene was found in the C&L Inventory database (database contains classification and labelling information on notified and registered substances received from manufacturers and importers; http://www.echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database).

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Not available.

RAC general comment
Isopropyl (2E,4E,7S)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate (common name S-methoprene) is a biocidal active substance approved for use in biocidal products (type 18) as stated in Regulation (EC) 91/2014 of 31 January 2014. S-methoprene is not currently classified according to the CLP Regulation (EC) No. 1272/2008.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

S-Methoprene is a biocidal active substance approved for use in biocidal products for product type 18 from 1 September 2015 as stated in Regulation (EC) 91/2014 of 31 January 2014. The classification and labelling proposal includes environmental toxicity endpoints and needs to be evaluated under the CLP Regulation (EC) No. 1272/2008

Part B.

SCIENTIFIC EVALUATION OF THE DATA

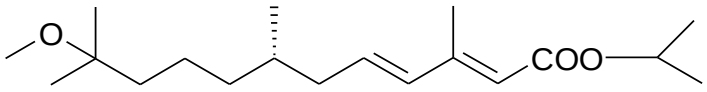
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	Not available
EC name:	Not available
CAS number (EC inventory):	Not available
CAS number:	65733-16-6
CAS name:	Not available
IUPAC name:	Isopropyl (2E,4E,7S)-11-methoxy-3,7,11-trimethyldodeca- 2,4-dienoate
CA Index Name	2,4-Dodecadienoic acid, 11-methoxy-3,7,11-trimethyl-1-methylethyl ester, (2E,4E,7S)-
CLP Annex VI Index number:	Not available
Molecular formula:	$C_{19}H_{34}O_3$
Molecular weight range:	310.48 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
S-Methoprene	95%	-	-

Current Annex VI entry:

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
-	-	-	-
All impurities have been claimed confidential			

Current Annex VI entry:

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
-	-	-	-	-

Current Annex VI entry:

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: Summary of physico-chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Freezing point (state purity)	Purity: 98.3% < -22°C	Laky, V., 2006a	
Boiling point (state purity)	Purity: 99.6% 279.9 °C	Laky, V., 2006a	
Temperature of decomposition	Not applicable as the boiling point was estimated.		
Appearance (state purity)	Purity: > 95% A transparent pale yellow liquid at 24°C with a faint, fruity, waxy odour.	Anderson, W., 1999	

Relative density (state purity)	Purity: > 95% 0.924 g/ml at 20°C	Anderson, W., 1999	
Surface tension	Purity: 98.3% 50.1 mN/m at 20°C (1mg/l)	Laky, V., 2006f	
Vapour pressure (in Pa, state temperature)	Purity: 98.1 % 0.623 mPa at 20°C 1.08 mPa at 25°C	Bates, M., 2007	
Henry's law constant (Pa m^3 mol^{-1})	0.0306 Pa x m^3/mol at 20°C	Bates, M., 2007	
Solubility in water (g/l or mg/l, state temperature)	Purity: > 95% 6.85 mg/l at 20 °C	Anderson, W., 1999	
Solubility in organic solvents (in g/l or mg/l, state temperature) (Annex IIIA, point III.1)	Purity: 98.1% Hexane: > 5 10^5 mg/l Methanol: > 4.5 10^5 mg/l Acetone: > 5 10^5 mg/l Temperature: 20 ± 1 °C	Laky, V., 2006a	
Stability in organic solvents used in biocidal products including relevant breakdown products (IIIA, point III.2)	Not required as no organic solvents are present in the technical.	Laky, V., 2006a	
Partition coefficient (log P_{OW}) (state temperature)	LogKow = 6.34	Rivendell International 2012	Calculated
Hydrolytic stability (DT_{50}) (state pH and temperature) (point VII.7.6.2.1)	pH 1.2: 17 hours at 37 ± 0.5°C	Anderson, W., 1999	
	pH 4: Stable at 25 ± 0.5°C, 37 ± 0.5°C and 50 ± 0.5°C	Laky, V. (2002a),	
	pH 7: Stable at 25 ± 0.5°C, 37 ± 0.5°C and 50 ± 0.5°C		
	pH 9: Stable at 25 ± 0.5°C, 37 ± 0.5°C and 50 ± 0.5°C		
Dissociation constant (not stated in Annex IIA or IIIA; additional data requirement from TNsG)	Not required as S-Methoprene does not dissociate in water.		
UV/VIS absorption (max.) (if absorption > 290 nm state ε at wavelength)	Purity: 95% <u>90% Neutral Methanol:</u> λ_{max} 264 nm; ε 26,700 <u>90% Acidified Methanol:</u> λ_{max} 264 nm; ε 26,600	Anderson, W., 1999	

	90% Alkalinized Methanol: λ_{max} 266 nm; ε 27,450		
Flammability	Purity: 98.3% 263 °C	Laky, V., 2006d	
Explosive properties	The molecular structure of S-Methoprene indicates that the substance has little or no explosive properties.		

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed no classification.

Comments received during public consultation

One Member State Competent Authority (MSCA) requested further clarifications regarding oxidising and explosive properties of S-methoprene. The DS responded that the molecular structure of S-methopene does not predict oxidising or explosive properties.

Assessment and comparison with the classification criteria

The molecular structure of S-methoprene indicates that the substance has little or no explosive properties and the other physico-chemical properties do not raise alerts. Thus, **RAC agrees with the proposal of the DS for no classification of S-methoprene for physical hazards.**

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Insecticide (PT 18) – Biocide.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
-	-	-	-
-	-	-	-
-	-	-	-
-	-	-	-

3.1 *[Insert hazard class when relevant and repeat section if needed]*

3.1.1 Summary and discussion of S-Methoprene

Not applicable. S-Methoprene does not classify with respect to physical chemistry.

3.1.2 Comparison with criteria

No classification is warranted for S-Methoprene regarding physic-chemical hazardous properties based on study results summarised in Table 9 above.

3.1.3 Conclusions on classification and labelling

No classification is warranted for S-Methoprene with respect to physical chemistry.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

In a toxicokinetic study, [^{14}C]-S-Methoprene was administered to male and female Sprague-Dawley rats, and its excretion, absorption, distribution and metabolism were examined. The study consisted of four groups: a single low level dose group (25 mg/kg bw), a single high level dose group (250 mg/kg bw), a low level repeat dose group (seven daily oral doses of 25 mg/kg bw), and a bile duct cannulated group (single administration at 25 mg/kg bw).

All rats survived the study and no signs of toxicity were observed. Measurement of radioactivity in the plasma revealed that in the low dose group the peak plasma concentrations for males and females were at 6 and 12 hours respectively. In the high dose group, the peak plasma concentrations were seen at 4 hours in males and 6 hours in females.

The majority of [^{14}C]-S-Methoprene was excreted within 24 to 48 hours of administration, indicating that it is rapidly eliminated from the body. The primary routes of excretion of the compound were in the faeces and expired air, with a lesser amount recovered in urine and cage rinses.

For all dosing regimens, the tissue radioactivity was negligible at 96 hours after administration, with the exception of the white fat, which contained up to 4.633 % of the administered radioactivity. This is in line with previous reports suggesting that S-Methoprene is a lipophilic compound. However, in all other tissues the levels of radioactivity decreased between the 6 and 96 hour time points, indicating that neither the compound nor its metabolites accumulate in these tissues.

In all dosing regimens, males excreted a higher percentage of the [^{14}C]-S-Methoprene-derived radioactivity in the faeces than females. Sex differences in the tissue distribution of radioactivity were observed at the low level dose of [^{14}C]-S-Methoprene. Female tissue contained higher radioactivity than male tissue in this group.

The recovery of radioactivity following administration of [14C]-S-Methoprene to rats indicated that the majority of radioactivity (40-60 % AR) was excreted via the faecal route and the majority of this was the unchanged parent compound [^{14}C]-S-Methoprene (Quotient Bioresearch Report LIH/02). Of the remaining radioactivity 14-28 % AR was excreted via expired air indicating extensive metabolism of the parent molecule and incorporation of the radiolabel into endogenous components.

Based on the available evidence it is concluded that the metabolism of S-Methoprene proceeds via incorporation into natural products. Definitive identification of radioactive metabolites is rendered almost impossible by the high background observed from unlabelled natural components already in the circulation. The presence of S-Methoprene was confirmed using mass spectrometric techniques, whilst the presence of Methoprene acid, acetate and glycine were determined using co-chromatography with unlabelled or radiolabelled reference materials. The only component in rat excreta samples that represented > 10 % of administered radioactivity was RF23 and this component was shown to be the parent compound S-Methoprene.

Previous studies summarised by the World Health Organisation (WHO) (1998 and 1999) reached similar conclusions regarding the metabolism of Methoprene in animals. In the current study both S-Methoprene and S-Methoprene acid could be detected using GC-MS analysis. S-Methoprene was detected in faeces, whilst no other metabolites were detected. It would be expected that any components with structures similar to S-Methoprene would have been detected if present in the samples. The WHO document references metabolism in large animals (Cow, Steer and Hen) whereas the current study was performed in the rat. Generally, the smaller the animal species the higher the rate of metabolic turnover. The results are therefore consistent with rapid degradation/metabolism of Methoprene into C-1 and C-2 units and fully consistent with previously published data on the metabolism of Methoprene in the rat. In the current study there was one component (RF23, identified as S-Methoprene) which was greater than 10 % dose and four components (RF21, RF20, RF13 and RF7) that were greater than 5 %. These components were not identified in the current study but it should be noted that these were present in the faeces and may arise from unabsorbed material travelling through the GI tract following an oral dose and will therefore be of no toxicological concern.

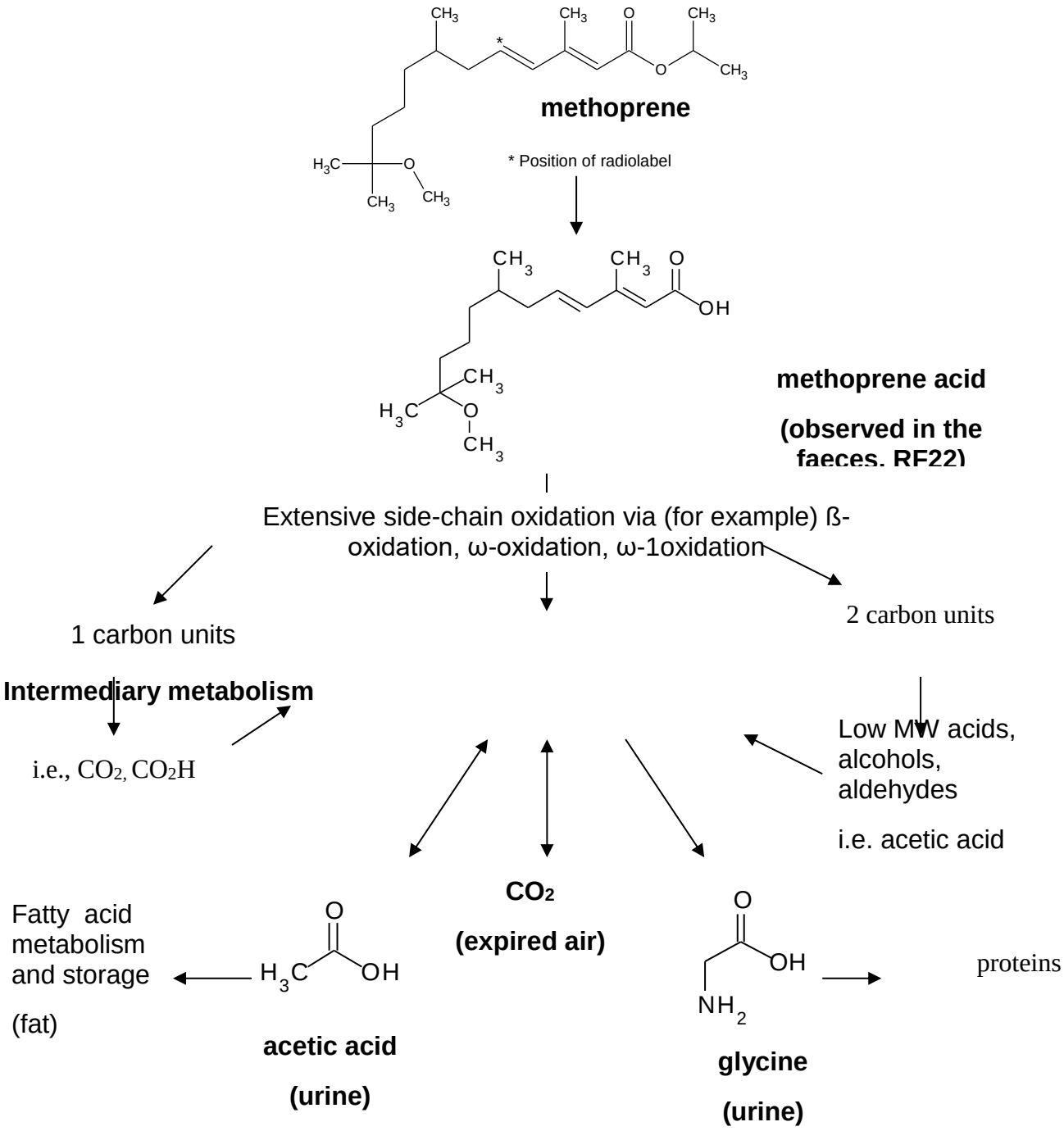
It is concluded that the metabolism of S-Methoprene proceeds via extensive degradation of the aliphatic chains and this may lead to production of saturated and/or unsaturated aliphatic, alicyclic linear primary alcohols, aldehydes and acids as intermediates to the ultimate production of acetate.

In addition, a literature search has been conducted to provide basic toxicokinetic data for S-Methoprene. The bio-kinetics and metabolism of Methoprene were investigated in various mammalian species such as rat and guinea pig following a single oral administration showing that

Methoprene is rapidly absorbed and excreted in urine, faeces and expired air. S-Methoprene is metabolised as a methyl branched fatty acid food and also via conjugation (O-dealkylation). Following treatment with glucuronidase, the two major metabolites in urine were 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoic acid and 11-hydroxy-3,7,11-trimethyldodeca-2,4-dienoic acid and accounted for 75% of the radiolabel in urine. 77% of the recovered radiolabel represented intact Methoprene in the faeces.

An overall value for oral absorption to be used in the risk assessment (35%) was derived from the basic toxicokinetics studies.

Proposed metabolic pathway for [^{14}C]-S-Methoprene



Dermal Absorption

A study investigating the rate and extent of absorption of S-Methoprene following topical application of a single formulation of the test substance to human skin was conducted. The mean percentage recovery of the applied test substance was 100±10%. The mean percentage found in the receptor fluid was 0.04%, the mean percentage found remaining in the skin was 1.61%, the mean percentage found in the stratum corneum (tape strips 6-20) was 1.21% and the mean percentage found remaining unabsorbed was 97.14% at 24 hours. As strips 3-5 were also considered part of the potentially

absorbed dose an additional 0.58% was added to the value calculated by the Notifier (2.86%). From this study it was determined the dermal absorption of [^{14}C] -S-Methoprene is 3.44 ≈ 3.5%.

4.1.1 Non-human information

4.1.2 Human information

None available.

4.1.3 Summary and discussion on toxicokinetics

[^{14}C]-S-Methoprene was administered to male and female Sprague-Dawley rats, and its excretion, absorption, distribution and metabolism were examined. Measurement of radioactivity in the plasma revealed that in the low dose group the peak plasma concentrations for males and females were at 6 and 12 hours respectively. In the high dose group, the peak plasma concentrations were seen at 4 hours in males and 6 hours in females.

The majority of [^{14}C]-S-Methoprene was excreted within 24 to 48 hours of administration, indicating that it is rapidly eliminated from the body. The primary routes of excretion of the compound were in the faeces and expired air, with a lesser amount recovered in urine and cage rinses.

For all dosing regimens, the tissue radioactivity was negligible at 96 hours after administration, with the exception of the white fat, which contained up to 4.633 % of the administered radioactivity.

S-Methoprene is metabolised as a methyl branched fatty acid food and also via conjugation (O-dealkylation). Following treatment with glucuronidase, the two major metabolites in urine were 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoic acid and 11-hydroxy-3,7,11-trimethyldodeca-2,4-dienoic acid and accounted for 75% of the radiolabel in urine. 77% of the recovered radiolabel represented intact Methoprene in the faeces.
An overall value for oral absorption to be used in the risk assessment (35%) was derived from the basic toxicokinetics studies.

The dermal absorption of [^{14}C]-S-Methoprene was determined in an in-vitro dermal absorption study to be 3.44 ≈ 3.5%.

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
OECD 401/US EPA 81-1. Rat HSD;SD 5050 mg/kg bw Single oral dose)	**Value** **LD_{50} / LC_{50}**> 5050 mg/kg. S-Methoprene does not require classification.	No deaths occurred at the 5050 mg/kg dose level. Clinical observations included crust around the nose, piloerection, diarrhoea, activity decrease and an oily yellow substance at the base of the tail, all of which were no longer evident by Day 6 of the study. Body weight gain remained unaffected. Terminal necropsy revealed no abnormalities	Kuhn, J. O. (1999a) S-Methoprene CAR IIIA 6.1.1
OECD 402/US EPA 81-1.(Rabbit Albino New Zealand White Male / Female 5/sex 5050mg/kg bw Single dermal dose)	**Value** **LD_{50} / LC_{50}**> 5050 mg/kg. S-Methoprene does not require classification.	Erythema was observed on Day 1, and no longer evident on Day 4. Signs of dermal irritation including erythema and desquamation were observed on Day 1 but were no longer evident on Day 4. Body weight gain was unaffected by the administration of S-Methoprene. Terminal necropsy revealed no observable abnormalities	Kuhn, J. O. (1999b) S-Methoprene CAR IIIA 6.1.2
OECD 403/US EPA 81-3. Rat 2.38 mg/l	**Value** **LD_{50} / LC_{50}** > 2.38 mg/L S-Methoprene does not require classification.	Clinical signs of toxicity included activity decrease and piloerection in both sexes. Red staining around the nose in males. Animals were asymptomatic by Day 1. No mortalities were recorded. Terminal necropsy revealed no observable abnormalities with the exception of discoloured lungs and a swollen large intestine in one male and discoloured lungs in two females. Body weight gain was generally unaffected by administration of the test substance however, one male failed to gain weight and one female lost weight during the first week.	Leeper, L. (1999) S-Methoprene CAR IIIA 6.1.3

*New studies submitted for the Biocides Review (2013).

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Kuhn, J.O. (1999a), Acute oral toxicity study in rats: The acute oral toxicity of S-Methoprene was investigated in Harlan Sprague Dawley rats. S-Methoprene was administered by oral gavage to male

and female rats (5/sex) at a dose level of 5050 mg/kg. The study is comparable to OECD guideline 401. No deaths occurred at the 5050 mg/kg dose level. Clinical observations included crust around the nose, piloerection, diarrhoea, activity decrease and an oily yellow substance at the base of the tail, all of which were no longer evident by Day 6 of the study. Body weight gain remained unaffected. Terminal necropsy revealed no abnormalities.

Based on the results from the acute exposure oral toxicity study in rats, the acute oral LD_{50} for S-Methoprene was determined to be > 5050 mg/kg. The acute oral LD_{50} of S-Methoprene in rats was determined to be greater than 5050 mg/kg.

In accordance with the provisions of CLP Regulation (EC) No. 1272/2008, S-Methoprene does not require classification for acute oral toxicity.

4.2.1.2 Acute toxicity: inhalation

Leeper, L. (1999), Acute inhalation toxicity study in rats. The acute inhalation toxicity of S-Methoprene in Sprague Dawley rats was investigated by administering a single dose of S-Methoprene to one group of 5/sex rats as a liquid aerosol by the inhalation route for an exposure duration of four hours and at a concentration of 2.38 mg/L. The study is comparable to OECD 403.

Clinical signs of toxicity included activity decrease and piloerection in both sexes and red staining around the nose in males. Animals were asymptomatic by Day 1. No mortalities were recorded throughout the study period. Terminal necropsy revealed no observable abnormalities with the exception of discoloured lungs and a swollen large intestine in one male and discoloured lungs in two females. Body weight gain was generally unaffected by administration of the test substance however, one male failed to gain weight and one female lost weight during the first week.

Table 12: Mortality data following inhalation of S-Methoprene technical

Dose (mg/ L)	Males Mortality	Time of death – hrs (no of animals)	Dose (mg/L)	Females Mortality	Time of death – hrs (no of animals)
2.38	0/5	-	2.38	0/5	-

The acute inhalation LC_{50} of S-Methoprene in male and female albino SD rats was determined to be greater than 2.38 mg/L. In accordance with the provisions of CLP Regulation (EC) No. 1272/2008, S-Methoprene does not require classification for acute inhalation toxicity.

4.2.1.3 Acute toxicity: dermal

Kuhn, J. O. (1999b), Acute dermal toxicity study in rabbits. The acute dermal toxicity of S-Methoprene was investigated by applying a single dose of S-Methoprene at a concentration of 5050 mg/kg to the skin of 5 albino New Zealand white rabbits/sex. The study is comparable to OECD guideline 402 and is described. Clinical observations revealed soft faeces at 1 and 4 hrs exposure to S-Methoprene in the male rabbit but this reaction was no longer evident by Day 1 of the examination period. Erythema was observed on Day 1, and no longer evident on Day 4. Signs of dermal irritation including erythema and desquamation were observed on Day 1 but were no longer evident on Day 4.

Body weight gain was unaffected by the administration of S-Methoprene. Terminal necropsy revealed no observable abnormalities.

Table 13: Acute Dermal Toxicity in the Rabbit (Limit Test): Mortality Data

Dose (mg/ kg)	Males Mortality	Time of death – hrs (no of animals)	Dose (mg/kg)	Females Mortality	Time of death – hrs (no of animals)
5050	0/5	-	5050	0/5	-

The dermal LD_{50} of S-Methoprene in rabbits was determined to be greater than 5050 mg/kg. In accordance with the provisions of CLP Regulation (EC) No. 1272/2008, S-Methoprene does not require classification for acute inhalation toxicity.

4.2.1.4 Acute toxicity: other routes

No data.

4.2.2 Human information

No data.

4.2.3 Summary and discussion of acute toxicity

Conclusion: The acute oral LD_{50} and dermal LD_{50} of S-Methoprene in rats were determined to be greater than 5050 mg/kg. The acute inhalation LC_{50} of S-Methoprene in rats was found to be greater than 2.38mg/l. Clinical signs of intoxication were non-specific and reversible (piloerection, diarrhoea, decreased activity and some dermal irritation in the dermal study). All studies were carried out using protocols comparable to accepted guidelines and according to GLP (self-certified). In accordance with the provisions of CLP Regulation (EC) No. 1272/2008, S-Methoprene remains unclassified and requires no symbols or risk phrases for acute oral, dermal and inhalation toxicity.

4.2.4 Comparison with criteria

The classification criteria for the acute oral, dermal or inhalation toxicity of S-methoprene are not met.

4.2.5 Conclusions on classification and labelling

Taking the rat oral data, (greater than 5050 mg/kg bw/day) S-Methoprene does not classify for acute oral toxicity when compared to the requirements of the CLP Regulation (EC) No. 1272/2008. Equally, dermal or inhalation exposure study results do not warrant classification under CLP Regulation (EC) No. 1272/2008.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of S-methoprene for acute toxicity on the basis of studies (one per route of exposure) yielding LD_{50} values greater than 5050 mg/kg bw for the oral and dermal routes and higher than 2.38 mg/L for inhalation.

Comments received during public consultation

No comments were received during public consultation on this hazard class.

Assessment and comparison with the classification criteria

The table below summarises acute oral, dermal and inhalation toxicity studies that were reported in the CLH report.

METHODOLOGY	RESULTS AND REMARKS	REFERENCE
OECD TG 401/US EPA 81-1 Rat HSD;SD 5 animals/sex Single dose (gavage): 5050 mg/kg bw	No mortalities. Clinical observations: crust around the nose, piloerection, diarrhoea, activity decrease and an oily yellow substance at the base of the tail, none of which were any longer evident by Day 6 of the study. Body weight gain remained unaffected. Terminal necropsy revealed no abnormalities. **LD_{50} > 5050 mg/kg bw**	Kuhn, 1999a Document IIIA 6.1.1 in S-methoprene CAR
OECD TG 402/US EPA 81-1. Rabbit Albino New Zealand White 5 animals/sex Single dermal dose: 5050 mg/kg bw	No mortalities. Clinical observations: signs of dermal irritation including erythema and desquamation were observed on Day 1 but were no longer evident on Day 4. Body weight gain remained unaffected. Terminal necropsy revealed no abnormalities. **LD_{50} > 5050 mg/kg bw**	Kuhn, 1999b Document IIIA 6.1.2 in S-methoprene CAR
OECD TG 403/US EPA 81-3. Sprague Dawley rats 5 animals/sex	No mortalities Clinical observations: activity decrease and piloerection in both sexes, red staining around the nose in males. Animals were asymptomatic by Day 1.	Leeper, 1999 Document IIIA 6.1.3 in S-methoprene CAR

4 hours of exposure to a liquid aerosol Single dose of 2.38 mg/L	Terminal necropsy revealed discoloured lungs and a swollen large intestine in one male and discoloured lungs in two females. One male failed to gain weight and one female lost weight during the first week. **LC_{50} > 2.38 mg/L**	

The cut-off values for classification for both oral and dermal routes are 2000 mg/kg bw and S-methoprene was concluded to have LD_{50} values by both routes well above 5050 mg/kg bw. The cut-off value for classification for the inhalation route is 5 mg/L and S-methoprene was concluded to not cause mortalities after exposures to 2.38 mg/L. This suggests that it is unlikely that the LC_{50} is lower than 5 mg/L, especially considering that the clinical signs observed after inhalation exposures to 2.38 mg/L S-methoprene were reversible.

In conclusion, **RAC** agrees with the DS's proposal and considers that S-methoprene **does not meet the criteria for classification for acute toxicity by any route of exposure.**

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

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

There is no indication from the dossier presented that specific target organ toxicity will result from a single exposure.

4.3.2 Comparison with criteria

Not applicable.

4.3.3 Conclusions on classification and labelling

There is no indication from the dossier presented that specific target organ toxicity will result from a single exposure.

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

Summary of the Dossier Submitter's proposal

The DS proposed no classification for STOT SE because there were no indications that S-methoprene induced specific target organ toxicity after a single exposure.

Comments received during public consultation

No comments were received during public consultation on this hazard class.

Assessment and comparison with the classification criteria

According to the CLP Regulation, specific target organ toxicity following a single exposure should be considered where there is clear evidence of toxicity to a specific organ, especially when it is observed in the absence of lethality. The standard acute toxicity studies do not indicate that there is any specific organ toxicity following a single exposure. Overall, it is concluded that classification of S-Methoprene for STOT SE 1 or 2 is not warranted.

The hazard class STOT SE 3 covers 'transient' respiratory tract irritation and narcotic effects that are observed in animal studies and that may include lethargy, lack of coordination, loss of righting reflex and ataxia occurring after single exposure. None of these effects were reported in the available acute toxicity studies.

RAC therefore agrees with the DS, that classification **of S-methoprene for STOT SE is not warranted.**

4.4 Irritation

4.4.1 Skin irritation

Table 14: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation in NZW rabbits. S-Methoprene OECD 404	Non-irritating	-	Kuhn, J.O. (1999c) S-Methoprene CAR IIIA 6.1.4/1

4.4.1.1 Non-human information

Kuhn, J.O. (1999c), Primary Dermal Irritation Study In Rabbits. In a primary dermal irritation study, 3 young adult (2 males and 1 female) white albino New Zealand rabbits were dermally exposed to 0.5 mL S-Methoprene technical (LX 125-03) for a single 4-hour application to one intact site on each animal. The trunk of the animals was wrapped with a semi-permeable dressing and secured with strips of tape to retard evaporation and prevent possible ingestion of the test substance. At the end of the exposure period, the wrappings were removed and the skin was gently wiped with water and a clean cloth to remove any residual test substance. The acute dermal irritation index was calculated and S-Methoprene was classified according to the Draize method.

Erythema was observed in all rabbits at the 1 hr observation period and in one male and female at the 24 hr observation period. All rabbits returned to normal at the 48 hour observation period. No oedema was observed in any of the animals at throughout the study. The average score for erythema and oedema for all animals at 24-72 hours was 0.11 and 0, respectively.

Table 15: Dermal Irritation scores following exposure to S-Methoprene

	Erythema						Oedema					
Animal no.	1	2	3	4	5	6	1	2	3	4	5	6
After 1 hr	1	1	1	1	1	1	0	0	0	0	0	0
After 24 hr	0	1	0	1	0	0	0	0	0	0	0	0
After 48 hr	0	0	0	0	0	0	0	0	0	0	0	0
After 72 hr	0	0	0	0	0	0	0	0	0	0	0	0
Mean score 24-72 hr	0.11						0.0					

On the basis of reactions observed in this study and the criteria defined in CLP Regulation (EC) No. 1272/2008, S-Methoprene does not require classification for dermal irritation.

4.4.1.2 Human information

Not available.

4.4.1.3 Summary and discussion of skin irritation

S-Methoprene was not irritating to the skin in the studies presented.

4.4.1.4 Comparison with criteria

The criteria for skin irritation are not met.

4.4.1.5 Conclusions on classification and labelling

S-Methoprene does not classify for skin irritation when compared to the requirements of the CLP Regulation (EC) No. 1272/2008.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for S-methoprene for skin irritation on the basis of a study performed in accordance with OECD Test Guideline (TG) 404 in which the average scores for erythema and oedema for all animals at 24-72 hours were 0.11 and 0.0, respectively.

Comments received during public consultation

No comments were received during public consultation on this hazard class.

Assessment and comparison with the classification criteria

Kuhn (1999c) (document IIIA 6.1.4/1 in the CAR for S-methoprene) studied the skin irritation of S-methoprene in a primary dermal irritation study in New Zealand White (NZW) rabbits performed according to OECD TG 404. Rabbits were dermally exposed to 0.5 mL

undiluted S-methoprene for 4 hours with a semi-permeable dressing secured with strips of tape. At the end of the exposure period, the wrappings were removed and the skin was gently wiped. The acute dermal irritation index was calculated according to the Draize method.

Erythema was observed in all rabbits at the 1 h observation period and in 1 male and 1 female at the 24 h observation period. The erythema had reversed in all rabbits within the 48 hours observation period. No oedema was observed in any of the animals throughout the study. The average scores for erythema and oedema for all animals at 24-72 hours were 0.11 and 0.0, respectively.

In conclusion, no oedema was recorded and the mean score over 24-72 hours for erythema was considerably lower than the minimum required by the CLP Regulation for classification for skin irritation. Therefore, in concordance with the DS, **RAC agrees that no classification for skin corrosion or irritation is warranted for S-methoprene.**

4.4.2 Eye irritation

Table 16: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation in NZW rabbits S-Methoprene OECD 405	Non-irritant	Slight reversible erythema.	Kuhn, J.O. (1999d) S-Methoprene CAR IIIA 6.1.4/2

4.4.2.1 Non-human information

Kuhn, J.O. (1999d), Primary eye irritation study in rabbits. The acute eye irritation of S-Methoprene was investigated by instilling a single dose (0.1 mL) of S-Methoprene technical (LX 125-03) into the conjunctival sac of one eye of a group of six New Zealand White rabbits (3/sex). The lids were thereafter gently held together for one second and then released. The left eyes served as controls. Following installation of S-Methoprene, the eyes of all animals were observed for signs of ocular

irritation at 1, 24, 48 and 72 hours after treatment. The grades of ocular reaction were recorded at each examination period.

Table 17: Eye Irritation Scores following exposure to S-Methoprene

	Corneal Opacity						Iridial Inflammation					
Time/Rabbit	**9224 M**	**9226 M**	**9230 M**	**9225 F**	**9227 F**	**9229 F**	**9224 M**	**9226 M**	**9230 M**	**9225 F**	**9227 F**	**9229 F**
1h	0	0	0	0	+	+	0	0	0	0	0	0
24h	0	0	0	0	0	0	0	0	0	0	0	0
48h	0	0	0	0	0	0	0	0	0	0	0	0
72h	0	0	0	0	0	0	0	0	0	0	0	0
Mean Score (24-72 hr)	0						0					

+ slightly dulling of normal luster

	Conjunctival Redness						Conjunctival Chemosis					
Time/Rabbit	**9224 M**	**9226 M**	**9230 M**	**9225 F**	**9227 F**	**9229 F**	**9224 M**	**9226 M**	**9230 M**	**9225 F**	**9227 F**	**9229 F**
1h	1	1	1	2	1	2	1	1	1	1	1	1
24h	0	0	0	1	0	1	0	0	0	0	0	0
48h	0	0	0	0	0	0	0	0	0	0	0	0
72h	0	0	0	0	0	0	0	0	0	0	0	0
Mean Score (24-72 hr)	0.11						0					

Average scores (24-72 hours) of 0 for cornea, 0 for Iris and 0 for Chemosis were recorded. Conjunctiva redness was scored at 0.11 for 24-72 hours. All effects had reversed at 48 hours and on the basis of reactions observed in this study and the criteria defined in CLP Regulation (EC) No. 1272/2008, S-Methoprene does not require classification for acute eye irritation.

4.4.2.2 Human information

Not available.

4.4.2.3 Summary and discussion of eye irritation

Slight reversible Conjunctiva redness was seen in the study presented. This was fully reversible within 48 hours.

4.4.2.4 Comparison with criteria

The criteria for eye irritation are not met.

4.4.2.5 Conclusions on classification and labelling

S-Methoprene does not classify for eye irritation when compared to the requirements of the CLP Regulation (EC) No. 1272/2008. Classification not warranted.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of S-methoprene for eye irritation on the basis of a study, performed in accordance with OECD TG 405, showing that the average scores for corneal opacity, inflammation of the iris, conjunctival redness and conjunctival chemosis over 24-72 hours were 0.0, 0.0, 0.11 and 0.0, respectively.

Comments received during public consultation

No comments were received during public consultation on this hazard class.

Assessment and comparison with the classification criteria

Kuhn (1999d) (document IIIA 6.1.4/2 in the CAR for S-methoprene) studied the eye irritation of S-methoprene in a primary eye irritation study in NZW rabbits, performed according to OECD TG 405. Rabbits were instilled in the right eye with 0.1 mL undiluted S-methoprene into the conjunctival sac. The lids were thereafter gently held together for one second and then released. The left eyes served as controls. Following instillation of S-methoprene, the eyes of all animals were observed and scored for signs of ocular irritation at 1, 24, 48 and 72 hours after treatment. The results were as follows:

- No corneal opacity was found in any of the six animals at any of the tested times. Thus, the mean score over 24-72 hours was 0.
- No inflammation of the iris was found in any of the six animals at any of the tested times. Thus, the mean score over 24-72 hours was 0.
- Six animals scored 1 for conjunctival chemosis at 1 hour after treatment; however, the observations performed 24, 48 and 72 hours after treatment were always 0 for the six animals. Thus, the mean score over 24-72 hours was 0.
- Three males and one female scored 1 and two females scored 2 for conjunctival redness at 1 hour after the treatment. These same two females scored 1 at 24 hours after treatment, while the other four animals scored 0. All animals scored 0 at the observations performed at 48 and 72 hours after treatment. The mean score over 24-72 hours was 0.11.

RAC notes that the reported effects were always mild and reversible and that the mean scores did not reach the minimum values for warranting classification as eye irritant category 2 (1.0 for corneal opacity and iridial inflammation and 2.0 for conjunctival redness and chemosis). Consequently, **RAC agrees with the proposal of the DS that S-methoprene does not warrant classification for eye irritation.**

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

S-Methoprene is not classifiable for skin or eye irritation and respiratory tract irritation is not anticipated. There was no evidence of respiratory tract irritation in the acute studies provided.

4.4.3.2 Human information

Not available.

4.4.3.3 Summary and discussion of respiratory tract irritation

Not indicated.

4.4.3.4 Comparison with criteria

Not applicable.

4.4.3.5 Conclusions on classification and labelling

Not applicable.

4.5 Corrosivity

Table 18: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference

4.5.1 Non-human information

S-Methoprene is not irritating to the skin or eyes in the rat. Corrosivity not relevant.

4.5.2 Human information

Not available.

4.5.3 Summary and discussion of corrosivity

4.5.4 Comparison with criteria

Based upon the irritation studies submitted for S-Methoprene, discussed in Section 4.4.1 and 4.4.2 above, corrosivity is not anticipated.

4.5.5 Conclusions on classification and labelling

No classification.

4.5.6 Skin sensitisation

Table 19: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Beuhler Method. (OECD 406). Hartley guinea pigs	No sensitisation reaction	-	Kuhn, J. O. (1999e) CAR IIIA 6.1.5

4.5.6.1 Non-human information

Kuhn, J.O. (1999e), Dermal Sensitization Study In Guinea Pigs. In a dermal sensitization study with S-Methoprene technical (LX 125-03; 97.2%), 10 hartley-albino guinea pigs (5 animals/sex) were tested using the method of Buehler for a total of three six-hour insult periods. Induction treatments were on Days 1, 8 and 15. Observations for skin reactions were made approximately 24 hours and 48 hours after the first induction treatment and 48 hours after each subsequent treatment. Skin reactions were graded according to the Buehler scale. In a preliminary dose-range-finding study, 4 animals each (2/sex) were exposed to four different concentrations of S-Methoprene to determine the mildly irritating and highest non-irritating dose (100% v/v, 75% v/v, 50% v/v, 25% v/v in acetone). Based upon the results of the dose-range-finding studies, the test article was dosed as received for induction and challenge

Fourteen days after the last induction period, all test animals were challenged with 0.4 mL of undiluted test substance in the same manner as the induction treatments at a virgin test site on the right rear quadrant of the animal. A group of 10 naïve control (5/sex) animals were treated with S-Methoprene in the same manner. This group served as the control challenge group. Challenge application lasted 6 hours, as before. Observations were performed 24 hours and 48 hours after the challenge treatments.

Table 20: Summary of Skin Sensitization Results following Challenge Phase

	Number of animals with signs of allergic reactions / number of animals in group

	Naive control	Test group (S-Methoprene)	Positive control (2-Mercapto-Benzothiazole)
Scored after 24h	0/10	0/10	9/10
Scored after 48h	0/10	0/10	8/10

The test substance, S-Methoprene, produced no irritation in naive control group animals after the single treatment at challenge. Similarly, the test substance produced no signs of irritation in test group animals after the challenge treatment and therefore did not elicit a sensitising reaction in guinea pigs. In accordance with the criteria defined in CLP Regulation (EC) No. 1272/2008, S-Methoprene does not require classification for skin sensitization.

4.5.6.2 Human information

Not available.

4.5.6.3 Summary and discussion of skin sensitisation

Dermal sensitisation was investigated in an acceptable test the "Beuhler test". There was no response consistent with dermal sensitisation.

4.5.6.4 Comparison with criteria

The criteria for skin sensitisation are not met.

4.5.6.5 Conclusions on classification and labelling

No sensitisation effects were detected in the Buehler test and therefore classification of S-Methoprene for skin sensitisation is not warranted.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of S-methoprene for skin sensitisation on the basis of a Buehler test, performed in accordance with OECD TG 406, showing that challenge with 0.4 mL of undiluted S-methoprene in animals previously induced using topical exposure on days 1, 8 and 15 also with undiluted S-methoprene caused no signs of allergic reactions.

Comments received during public consultation

One MSCA considered that the negative results of the Buehler test (3 applications) is not sufficient to conclude on the sensitising potential of the substance. The DS responded that there is no reason to doubt the validity of this test, because the test was conducted according to U.S. EPA Guideline 81-6, which is equivalent to OECD TG 406 and because the test substance produced no irritation in the naive control group or in challenged animals after the single treatment at challenge.

Assessment and comparison with the classification criteria

Kuhn (1999e) (document IIIA 6.1.5 in the CAR for S-methoprene) studied the skin sensitisation of S-methoprene in a skin sensitisation study in Guinea pigs performed according to OECD TG 406. In this study, 10 animals (5 animals/sex) were induced on days 1, 8 and 15 with undiluted S-methoprene. Fourteen days after the last induction period, all test animals were challenged with 0.4 mL of undiluted test substance. A group of 10 naive control (5/sex) animals were treated with S-methoprene in the same manner. The test substance produced no irritation 24 and 48 hours after challenge in any of the animals exposed in either the naive control or test group. However, 90% and 80% of the animals showed signs of allergic reactions in the positive control groups challenged with 2-mercapto-benzothiazole at 24 and 48 hours, respectively.

In conclusion, undiluted S-methoprene failed to induce skin sensitisation and therefore **RAC agrees** with the DS, that **no classification of S-methoprene for skin sensitisation.**

4.5.7 Respiratory sensitisation

No data.

Table 21: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
-	-	-	-
-	-	-	-
-	-	-	-

4.5.7.1 Non-human information

No data.

4.5.7.2 Human information

No data.

4.5.7.3 Summary and discussion of respiratory sensitisation

No data.

4.5.7.4 Comparison with criteria

No data.

4.5.7.5 Conclusions on classification and labelling

Not applicable.

4.6 Repeated dose toxicity

Note: Methoprene is a terpenoid consisting of a racemic mixture of two enantiomers (R and S in a ratio of 1:1). The activity of Methoprene as a juvenile hormone is restricted to the S enantiomer (S-Methoprene). S-Methoprene is the active isomer in the racemic mixture and is present in a 1:1 ratio; the results obtained in the studies with Methoprene are therefore adjusted to give the S-Methoprene equivalent. The NOEL from studies carried out with Methoprene has been adjusted and the NOEL for S-Methoprene included below:

Table 22: Summary table of relevant repeated dose toxicity studies

Species/study/dose	Findings at LOAEL	GV (extrap.) CLP	Reference
Rat, Oral, 90 days, S-Methoprene technical (96%): 0, 200, 400 and 1000 mg/kg/day	200 mg/kg bw/day. Statistically significant changes in organ weight such as liver and kidneys NOAEL was not determined. NOAEL: < 200 mg/kg bw/day		Szakonyi, I.P. (2002) CAR IIIA 6.1.4/2
Dog, Oral 90 days, S-Methoprene technical (96%): 0, 100, 300 and 1000 mg/kg bw/day Daily (Capsule)	300 mg/kg bw/day in males and females. Gastrointestinal signs including thin faeces and diarrhoea and increase in liver weight in males and females and in ALP activity in females NOAEL: 100 mg/kg bw/day in males and females		Török, T. (2007)
Rat, Oral, 104 weeks, S-Methoprene technical (96%): 0, 10.9, 43.4 and 217 mg/kg/day	5000 ppm (equivalent to 217 mg/kg bw/day) for Methoprene 2500 ppm (equivalent to 108.5 mg/kg bw/day) for S-Methoprene - 5000 ppm (equivalent to 217 mg/kg bw/day) increased incidence of hepatic lesions (bile-duct proliferation and portal lymphocyte infiltration) in males and increased absolute and relative weights of the liver in females. NOAEL: 1000 ppm (equivalent to 43.4 mg/kg bw/day) for Methoprene 500 ppm (equivalent to 21.7 mg/kg bw/day) for S-Methoprene		Wazeter, F.X., Goldenthal E.I., Geil, R.G., Benson, B.W., Keller W.F. and Blanchard, G.L. (1975)

Species/study/dose	Findings at LOAEL	GV (extrap.) CLP	Reference
Mouse, Oral, 18 month Methoprene technical: 250, 1000 and 2500 ppm (equivalent to 32.7, 130.8 and 327 mg/kg bw/day, respectively) (Dietary)	2500 ppm (equivalent to 327 mg/kg bw/day) for S-Methoprene. Increases in focal accumulations of macrophages with brownish foamy cytoplasm in the liver, often associated with small necrotic foci and mononuclear inflammatory cells. NOAEL: **Carcinogenicity;** 2500 ppm (equivalent to 327 mg/kg bw/day) for Methoprene 1250 ppm (equivalent to 163.5 mg/kg bw/day) for S-Methoprene **Toxicity;** 1000ppm (equivalent to 130.8 mg/kg bw/day) for Methoprene 500 ppm (equivalent to 65.4mg/kg bw/day) for S-Methoprene 125 ppm (equivalent to 16.35 mg/kg bw/day) for S-Methoprene.		Wazeter, F.X., Goldenthal, E.I., Geil, R.G. and Benson B.W. (1975)

Note: Methoprene is a terpenoid consisting of a racemic mixture of two enantiomers (R and S in a ratio of 1:1). The activity of Methoprene is restricted to the S enantiomer (S-Methoprene). Since S-Methoprene is the active isomer in the racemic mixture and as they are present in a 1:1 ratio it is possible to extrapolate the results obtained in the studies with Methoprene to S-Methoprene

4.6.1 Non-human information

4.6.1.1 Repeated dose toxicity: oral

Study 1. Szakonyi, I.P. (2002) 90-day rat feeding study (CAR IIIA.6.4.1)

Table 23: Summary of main findings

Study	Main findings	
Rat, Oral, 90 days, S-Methoprene technical (96%): 0, 200, 400 and 1000 mg/kg/day	Bodyweight gain was reduced compared to controls in male rats at 1000 mg/kg/day. A statistically significant difference was noted between weeks 7 and 13. However, this difference was reversible as the values of treated group exceeded the controls at the end of the recovery period. Sporadic statistically significant decreases were noted at 400 mg/kg/day. From day 63 until the end of the recovery period, the mean body weight in males at 1000 mg/kg/day was statistically significantly lower than controls. No treatment related changes in haematological parameters were recorded. At 400 mg/kg/day, increased HTC was noted in both males and females along with decreased MCHC in females. These variations were not considered to be treatment related. At 1000 mg/kg/day, slight increases in RBC and HTC in males and increase HGB in both males and females were recorded. Decreases in MCHC were elicited in both sexes at 1000	Szakonyi, I.P. (2002)

	mg/kg/day. However, these variations were not biologically significant and were within the physiological range. Increase in the glucose concentration was recorded in all female dose groups. However, this increase was only deemed to be relevant at 1000 mg/kg/day. A treatment related effect was proposed. Other changes such as decrease in bilirubin in both sexes at 200 mg/kg/day and in males at 400 mg/kg/day, decrease in carbamide and creatinine in females at 200 and 1000 mg/kg/day, increase in cholesterol in females at 200, 400 and 1000 mg/kg/day were reported. At 400 and 1000 mg/kg/day, decreased AST activity in males and females was observed. During the recovery period, increase in albumin concentration in males at 1000 mg/kg/day was noted. However, these variations were not biologically significant and were within the physiological range. At necropsy, the female that died on day 77 displayed reddish mottled lungs and congestive liver. However this was deemed to be a consequence of the paragastric treatment. Macroscopic findings in the lungs (emphysema, pinprick-sized and point-like haemorrhages, reddish mottled colour) noted in the control and all treated groups were correlated to the extermination process or considered to be incidental. Other findings such as enlarged, pale or nutmeg-like liver, pale or enlarged kidneys, pyelectasis, enlarged testes, smaller than normal thymus, haemorrhages in the urinary bladder, ovarial cyst, hydrometra) are commonly reported in rats of this age. A treatment related increase in liver weight (absolute and relative to bodyweight and brain weight) was recorded in all treated groups in both males and females. Increased kidney weight (absolute and relative to the body and brain weight) was observed in all male treated groups and was considered to be dose related. Increase in absolute kidney weight was reported in female at 400 and 1000 mg/kg/day. A dose related increase in kidney weight relative to body weight was noted in all female treated groups. The kidney referred to the brain weight increased only in females at 1000 mg/kg/day. At the end of the recovery period, the relative kidney weight referred to the body weight remained significantly higher than in the control in male animals. Other changes in the organ weights including brain, spleen, adrenals, testes, epididymides, ovaries and uterus were considered to be individual or incidental findings. The increase of organ weight issues originated from the increased number of healthy active cells of the organ could be considered as an adaptational process. LO(A)EL: 200 mg/kg bw/day NO(A)EL: No value determined. The value is somewhere between 0 and 200 mg/kg bw/day but has not been determined.	

*P<0.05, ** p<0.01, ns- not significant.

Table 24: Summary of body weight data in males and females (g)

Days of Study	Male				Female			
	0 mg/kg/day (weight ± SD)	200 mg/kg/day (weight ± SD)	400 mg/kg/day (weight ± SD)	1000 mg/kg/day (weight ± SD)	0 mg/kg/day (weight ± SD)	200 mg/kg/day (weight ± SD)	400 mg/kg/day (weight ± SD)	1000 mg/kg/day (weight ± SD)
1	169.15 ± 5.43	170.30 ± 5.54	169.10 ± 5.53	165.25 ± 7.47	146.30 ± 5.52	144.80 ± 5.81	146.60 ± 5.44	146.15 ± 6.48
7	229.95 ± 9.51	232.10 ± 10.61	231.00 ± 8.34	226.00 ± 7.40	172.35 ± 7.19	169.30 ± 7.13	167.30 ± 7.73	170.15 ± 9.86
14	286.30 ± 14.02	292.30 ± 17.86	290.50 ± 11.31	283.15 ± 11.37	195.45 ± 9.71	191.50 ± 7.52	191.50 ± 13.39	197.25 ± 13.19
21	329.95 ± 18.81	340.60 ±25.78	335.30 ± 16.81	327.35 ± 17.59	218.60 ± 11.86	212.90 ± 8.06	210.30 ± 16.10	219.60 ± 15.01
28	367.25 ± 23.09	376.00 ± 34.42	367.90 ± 21.48	360.40 ± 20.93	232.20 ± 13.04	228.80 ± 9.54	226.50 ± 17.84	235.55 ± 17.83
35	396.40 ± 27.03	403.80 ± 40.07	397.00 ± 26.12	387.40 ± 24.13	243.00 ± 12.37	239.80 ± 14.38	237.60 ± 14.83	246.15 ± 19.98
42	423.65 ± 30.43	439.00 ± 43.15	422.80 ± 28.40	411.25 ± 27.52	254.00 ± 14.90	251.80 ± 16.02	252.20 ± 19.63	260.15 ± 20.78
49	437.95 ± 33.37	456.20 ± 47.87[c]	433.70 ± 29.04	419.90 ± 30.75	260.70 ± 13.40	257.70 ± 13.12	256.50 ± 17.08	263.10 ± 20.93
56	457.05 ± 37.71	479.50 ± 49.36[d]	450.10 ± 32.92	432.70 ± 29.74	268.05 ± 14.10	267.00 ± 15.03	265.90 ± 14.67	272.20 ± 23.82
63	471.80 ± 39.81[a]	499.50 ± 54.18[d]	464.20 ± 38.44	441.80 ± 30.46	274.90 ± 15.82	273.60 ± 16.10	272.10 ± 15.95	279.00 ± 23.19
70	485.05 ± 39.83[a]	515.70 ± 59.45[e, d]	474.90 ± 39.36	451.30 ± 33.37	277.20 ± 15.98	276.50 ± 18.79	274.50 ± 15.34	281.75 ± 24.19
77	498.95 ± 40.02[a]	525.50 ± 58.84[e, d]	485.40 ± 39.72	458.10 ± 33.27	285.05 ± 16.65	285.50 ± 17.02	282.20 ± 18.81	288.15 ± 25.17
84	497.65 ± 39.69[b]	523.20 ± 58.19[d]	484.50 ± 38.07	456.95 ±34.81	275.80 ± 16.19	273.30 ± 16.49	271.70 ± 15.59	275.53 ± 26.77
89	508.65 ± 40.07[b]	531.30 ± 60.31 [e,d]	490.30 ± 40.23	461.00 ± 34.97	281.40 ± 17.81	275.40 ± 17.70	273.60 ± 15.72	284.68 ± 23.25
96	527.6 ± 37.00	-	-	481.7 ± 38.59	284.80 ± 15.25	-	-	296.3 ± 21.85
103	537.8 ± 36.21	-	-	486.6 ± 33.14	290.4 ± 15.83	-	-	294.4 ± 26.20
110	550.6 ± 40.29	-	-	505.5 ± 38.02	298.3 ± 16.35	-	-	303.8 ± 30.43
117	551.4 ±	-	-	511.3 ± 41.70	300.8 ±	-	-	310.0 ± 33.74

[a] p<0.05 and (group 4 - group 1) means group mean4 < group mean1
[b] p<0.01 and (group 4 - group 1) means group mean4 < group mean1
[c] p<0.05 and (group 4 - group 2) means group mean4 < group mean2
[d] p<0.01 and (group 4 - group 2) means

Table 25: Summary of terminal organ and body weights (g), organ/body weight and organ/brain weight ratios

Data collection	Sex	Group	Dose	Liver weight	Liver/ body weight	Liver/ brain weight	Kidney weight	Kidney/ body weight	Kidney/ brain weight
At study termination	Male	1M	0	11.50 ± 1.93	2.330 ± 0.216	526.09 ± 83.14	2.57 ± 0.24	0.524 ± 0.051	117.39 ± 7.85
		2M	200	14.39 ± 2.32[a]	2.751 ± 0.188[a]	631.23 ± 90.34[a]	3.11 ± 0.46[a]	0.598 ± 0.073[q]	136.22 ± 16.62[a]

		3M	400	14.37 ± 1.28[b]	3.016 ± 0.203[b, f]	669.69 ± 66.77[b]	3.25 ± 0.30[b]	0.683 ± 0.071[b, g]	151.12 ± 13.10[b, g]
		4M	1000	16.94 ± 1.57[c,d,e]	3.786 ± 0.373[c, d, e]	781.38 ± 70.63[c, d, e]	3.29 ± 0.23[c]	0.733 ± 0.046[c, d]	151.50 ± 9.34[c, p]
At the end of the recovery period		1M	0	10.88 ± 1.24	2.058 ± 0.260	481.94 ± 49.98	2.85 ± 0.24	0.537 ± 0.028	126.35 ± 9.78
		4M	1000	12.77 ± 1.13**	2.619 ± 0.225**	571.28 ± 46.26**	3.07 ± 0.30	0.627 ± 0.029**	137.23 ± 13.39
At study termination	Female	1F	0	6.87 ± 0.66	2.443 ± 0.186	341.16 ± 38.59	1.63 ± 0.17	0.581 ± 0.049	81.18 ± 10.15
		2F	200	7.90 ± 0.5[i]	2.885 ± 0.129**	390.54 ± 23.68**	1.75 ± 0.16	0.638 ± 0.044*	86.38 ± 7.01
		3F	400	9.71 ± 0.83[j, l]	3.572 ± 0.242**	464.35 ± 40.31**	1.86 ± 0.18[k]	0.683 ± 0.047**	88.81 ± 8.31
		4F	1000	13.45 ± 1.21[m, n, o]	4.800 ± 0.364**	663.94 ± 72.02**	2.20 ± 0.26[m, n, o]	0.789 ± 0.132**	108.19 ± 12.80[m, n, o]
At the end of the recovery period		1F	0	6.58 ± 0.72	2.322 ± 0.199	318.17 ± 35.01	1.72 ± 0.11	0.608 ± 0.028	83.09 ± 4.14
		4F	1000	7.32 ± 0.80	2.524 ± 0.178*	353.54 ± 35.94*	1.78 ± 0.15	0.614 ± 0.052	85.88 ± 7.86

* Statistically different from control, p<0.05
** Statistically different from control, p<0.01

Conclusion:

S-Methoprene treatment at 200, 400 and 1000 mg/kg/day resulted in a treatment related increase in liver weight (absolute and relative to bodyweight and brain weight) in all treated groups in both males and females. Increased kidney weight (absolute and relative to the body and brain weight) was observed in all male treated groups and was considered to be dose related. Increase in absolute kidney weight was reported in female at 400 and 1000 mg/kg/day. A dose related increase in kidney weight relative to body weight was noted in all female treated groups. At the end of the recovery period, the relative kidney weight referred to the body weight remained significantly higher than in the control in male animals. However, there was no evidence of specific organ toxicity outside increase in weight. Indeed, relative liver weights were 30% and 60% increased at the 400 mg/kg/day and 1000 mg/kg/day respectively without any noticeable impact on clinical chemistry or histological findings.

Study 2. Török, T. (2007) 90-day dog feeding study (CAR IIIA.6.4.1)

Dog 90-day, S-Methoprene technical (96%): 0, 100, 300 and 1000 mg/kg bw/day Daily (Capsule)	Gastrointestinal signs such as thin faeces and diarrhoea were observed in both sexes mostly in the 300 and 1000 mg/kg bw/day groups in a dose-related manner. There were no mortalities at any dose level during the study. No significant treatment related differences were found in body weights, food consumption, ophthalmoscopic examinations, haematology, urinalysis and macroscopic examinations. A statistically and biologically significant increase in ALKP activity was observed at the end of the midway and terminal period in males in the high dose group and in females in all treated groups. Enzyme activities were increased in a dose dependent manner but only exceeded historical control ranges in the higher dose groups. Increased ALKP activity is common although not specific

	in liver damage. The histopathological examination confirmed the suspicion of liver damage in the high dose groups. In both sexes in the 1000-mg/kg bw/day groups the weight of the livers showed a significant increase when compared to the control. In the 1000-mg/kg bw/day dose animals, a slight or medium degree of zonal vacuolisation of hepatocytes in the liver was observed. No other test material related lesions were detectable during the histopatholoy

Table 26: Summary of body weight data in males and females (kg)

Weeks of Study	Male				Female			
	0 mg/kg bw/day (weight ± SD)	100 mg/kg bw/day (weight ± SD)	300 mg/kg bw/day (weight ± SD)	1000 mg/kg bw/day (weight ± SD)	0 mg/kg bw/day (weight ± SD)	100 mg/kg bw/day (weight ± SD)	300 mg/kg bw/day (weight ± SD)	1000 mg/kg bw/day (weight ± SD)
1	7.93 ±	8.05 ± 0.37	8.05 ±	7.90 ±	8.73 ± 0.56	8.80 ± 0.47	8.73 ± 0.87	8.75 ±
2	8.33 ±	8.45 ± 0.33	8.33 ±	8.25 ±	9.03 ± 0.49	8.93 ± 0.44	9.08 ± 0.84	8.98 ±
3	8.65 ±	8.78 ± 0.25	8.63 ±	8.35 ±	9.18 ± 0.48	9.20 ± 0.22	9.30 ± 0.65	9.18 ±
4	8.95 ±	9.05 ± 0.19	8.90 ±	8.85 ±	9.33 ± 0.34	9.35 ± 0.17	9.48 ± 0.65	9.30 ±
5	9.20 ±	9.33 ± 0.26	9.10 ±	9.13 ±	9.68 ± 0.41	9.60 ± 0.16	9.73 ± 0.75	9.48 ±
6	9.45 ±	9.58 ± 0.13	9.55 ±	9.28 ±	9.98 ± 0.51	9.78 ± 0.19	9.90 ± 0.85	9.63 ±
7	9.50 ±	9.78 ± 0.22	9.53 ±	9.50 ±	10.28 ±	9.95 ± 0.21	9.90 ± 0.75	9.75 ±
8	9.63 ±	10.00 ± 0.28	9.58 ±	9.68 ±	10.38 ±	10.10 ±	10.03 ±	9.83 ±
9	9.88 ±	10.23 ± 0.31	9.80 ±	10.05 ±	10.53 ±	10.28 ±	10.15 ±	9.93 ±
10	9.98 ±	10.30 ± 0.29	9.95 ±	10.18 ±	10.60 ±	10.15 ±	10.25 ±	9.95 ±
11	10.00 ± 0.75	10.45 ± 0.31	10.18 ± 0.68	10.30 ± 0.83	10.73 ± 0.15	10.43 ± 0.15	10.40 ± 0.91	10.13 ± 1.00
12	10.15 ± 0.87	10.65 ± 0.40	10.33 ± 0.56	10.33 ± 0.92	10.90 ± 0.24	10.50 ± 0.22	10.53 ± 0.86	10.28 ± 1.00
13	10.20 ± 0.88	10.65 ± 0.52	10.33 ± 0.57	10.35 ± 0.84	10.93 ± 0.15	10.63 ± 0.33	10.53 ± 0.99	10.23 ± 0.93

Table 27: Summary of terminal organ and body weights (g), organ/body weight and organ/brain weight ratios

Data collection	Sex	Dose (mg/kg bw/day)	Liver weight (g)	Liver/ body weight (%)	Liver/ brain weight (%)
Terminal	Male	0	510.17 ± 111.65	5.090 ± 0.951	563.50 ± 95.26
		100	617.37 ± 111.37	5.836 ± 1.280	723.56 ± 154.51
		300	569.81 ± 95.34	5.690 ± 0.843	704.70 ± 114.96
		1000	731.21* ± 82.75	7.158* ± 0.595	860.99** ± 66.32

Terminal	Female	0	487.14 ± 64.64	4.451 ± 0.603	584.30 ± 101.87
		100	562.99 ± 83.26	5.304 ± 0.678	661.51 ± 92.66
		300	595.68 ± 116.92	5.704* ± 0.549	729.61 ± 152.62
		1000	678.19* ± 96.88	6.837** ± 0.913	836.46 ± 169.69

* Significantly different at 0.05 using Duncan's multiple range test
** Significantly different at 0.01 using Duncan's multiple range test

Conclusion:
S-Methoprene treatment at 100, 300 and 1000 mg/kg/day produced clinical signs such as thin faeces and diarrhea and increased liver weight in males at 1000 mg/kg (140% of controls) and females from 300 mg/kg (128% and 153%) and biologically and statistically significant increase in alkaline phosphatise (ALP) values in females from 300mg/kg bw/day (>10%). In addition, in the 1000-mg/kg bw/day dose animals, a slight or medium degree of zonal vacuolisation of hepatocytes in the liver was observed in both sexes. No other test material related lesions were detectable during the histopatholoy. Based on the findings found under the conditions of this study, the LOAEL was established to be 300-mg/kg bw/day and the NOAEL was established to be 100-mg/kg bw/day in beagle dogs.

Note: Study 3. Wazeter, F.X.,Goldenthal E.I., Geil, R.G., Benson, B.W., Keller W.F. and Blanchard, G.L. (1975) 104 week rat oral study (CAR IIIA.6.7.1) and Study 4 Wazeter, F.X., Goldenthal, E.I., Geil, R.G. and Benson B.W. (1975) 18-month mouse oral study are summarized in Section 4.9 of this document.

4.6.1.2 Repeated dose toxicity: inhalation

None available

4.6.1.3 Repeated dose toxicity: dermal

None available

4.6.1.4 Repeated dose toxicity: other routes

None available

4.6.1.5 Human information

None available

4.6.1.6 Other relevant information

None available

4.6.1.7 Summary and discussion of repeated dose toxicity

The toxicological properties of S-Methoprene upon short-term treatment were investigated in rat and dog. A sub chronic 90-day rat study (Szakonyi, I.P., 2002) did not provide an NOAEL value, and only an LOAEL of < 200 mg/kg was determined. The 90-day dog study(Torok, T., 2007) produced an NOAEL of 100mg/kg bw/day. The effects noted included clinical signs such as thin faeces and diarrhoea and increased liver weight in males at 1000 mg/kg (140% of controls) and females from 300 mg/kg (128% and 153%) and biologically and statistically significant increase inalkaline phosphatise (ALP) values in females from 300mg/kg bw/day (>10%). In addition vacuolization of hepatocytes were noted in both sexes at 1000mg/kg bw/day.

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

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

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

Classification for STOT Cat 2 is required when:

….on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

According to the CLP Regulation (EC) No. 1272/2008 S-Methoprene should not be considered for classification because clinical signs in the dog and liver effects in the dog and rat were seen at doses of 200 to 300 mg/kg bw/day. These doses are in excess of the cut-off value of 10≤100 mg/kg bw/day for Category 2 classification.

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

S-Methoprene does not require classification for STOT RE according to the CLP Regulation (EC) No. 1272/2008.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)
Summary of the Dossier Submitter's proposal The DS presented information from four different repeated dose toxicity studies, two 90-day oral studies (one in rats and one in dogs) and two carcinogenicity studies (one in rats and one in mice). The most relevant common finding in all these studies was that S-methoprene induced hepatotoxicity. This hepatotoxicity was manifested in the form of statistically significant organ weight changes at 200 and 300 mg/kg bw/d in the 90-day

studies in rats and dogs, respectively. These findings, together with increased incidences of hepatic lesions (bile-duct proliferation and portal lymphocyte infiltration and increases in focal accumulations of macrophages with brownish foamy cytoplasm) were also found at 108.5 and 163.5 mg/kg bw/d in the rat (104-weeks) and mouse (18-month) carcinogenicity studies, respectively. Other reported effects were also: i) increases in kidney weight at 300 mg/kg bw/d in the 90-day study in rats; and, ii) gastrointestinal alterations in the 90-day study in dogs at 300 mg/kg bw/d.

The DS proposed no classification of S-methoprene for STOT RE because the hepatotoxic and gastrointestinal effects all appeared at doses well-above the cut-off values for warranting classification for STOT RE.

Comments received during public consultation

No comments were received during public consultation on this hazard class.

Assessment and comparison with the classification criteria

The following table summarises the main relevant findings in the 90-day repeated dose toxicity studies and in the carcinogenicity studies.

METHOD	RESULTS	REMARKS
Rat Oral 90 days S-methoprene technical (96%): 0, 200, 400 and 1000 mg/kg bw/d	<u>1000 mg/kg bw/d:</u> Mean body weight statistically significantly reduced (around 9-10%) from day 63 in males. Clinical chemistry: Increases in glucose concentrations in females. <u>400 mg/kg bw/d:</u> Bodyweight gain statistically significantly and reversibly reduced between weeks 7 and 13. <u>All doses:</u> Increase in liver weight (absolute and relative to bodyweight and brain weight) in both males and females. Increase in kidney weight (absolute and relative to the body and brain weight) in males.	Szakonyi, 2002 Document IIIA 6.1.4/2 in S-methoprene CAR **Conclusion:** Increases in liver and kidney weight at doses of 200 mg/kg bw/d and higher. **Guidance value for classification as Category 2: 100 mg/kg bw/d**
Dog Oral 90 days S-methoprene technical (96%): 0, 100, 300 and 1000 mg/kg bw/d	<u>1000 mg/kg bw/d:</u> Gastrointestinal signs (thin faeces and diarrhoea). Increase in alkaline phosphatase activity in males. Liver lesions detected at histopathological examination.	Török, 2007 Document IIIA 6.1.4/2 in S-methoprene CAR **Conclusion:** Gastrointestinal and hepatic adverse effects at

Daily (capsule)	Significant increase in liver weight in males and females. Zonal vacuolation of hepatocytes in males and females. <u>300 mg/kg bw/d:</u> Gastrointestinal signs (thin faeces and diarrhoea). Increase in alkaline phosphatase activity in females.	doses of 300 mg/kg bw/d and greater. **Guidance value for classification as Category 2: 100 mg/kg bw/d.**
Rat Oral 104 weeks Methoprene technical (96% purity): 0, 10.9, 43.4 and 217 mg/kg bw/d	<u>217 mg/kg bw/d:</u> Increased incidence of hepatic lesions (bile-duct proliferation and portal lymphocyte infiltration) in males. Increased absolute and relative weights of the liver in females.	Wazeter *et al.*, 1975 Document IIIA 6.4.1 in S-methoprene CAR **Conclusion:** Hepatotoxicity at doses of 217 mg/kg bw/d of technical methoprene (equivalent to 108.5 mg/kg bw/d of S-methoprene)*. **Guidance value for classification as Category 2: 12.5 mg/kg bw/d.**
Mouse Oral 18 months Methoprene technical (96% purity): 32.7, 130.8 and 327 mg/kg bw/d, respectively Dietary	<u>327 mg/kg bw/d:</u> Increases in focal accumulations of macrophages with brownish foamy cytoplasm in the liver, often associated with small necrotic foci and mononuclear inflammatory cells.	Wazeter *et al*., 1975 Document IIIA 6.4.2 in S-methoprene CAR **Conclusion:** Hepatotoxicity at doses of 327 mg/kg bw/d of technical methoprene (equivalent to 163.5 mg/kg bw/d of S-methoprene)*. **Guidance value for classification as Category 2: 16.7 mg/kg bw/d.**
*Methoprene is a terpenoid consisting of a racemic mixture of two enantiomers (R and S in a ratio of 1:1). The activity of methoprene is restricted to the S enantiomer (S-methoprene). Since S-methoprene is the active isomer in the racemic mixture and as they are present in a 1:1 ratio it is possible to extrapolate the results obtained in the studies with methoprene to S-methoprene.		

Three different adverse effects were reported after repeated exposure to S-methoprene. These effects were:

- Gastrointestinal alterations at 300 mg/kg bw/d in the 90-day study in dogs.
- Increases in kidney weight at 200 mg/kg bw/d in the 90-day study in rats.
- Increases in liver weight at 200 and 300 mg/kg bw/d in the 90-day study in rats and dogs, respectively.
- Increased focal accumulations of macrophages with brownish foamy cytoplasm in liver at 163.5 mg/kg bw/d in the 18-months mouse carcinogenicity study.
- Bile-duct proliferation and portal lymphocyte infiltration in liver (together with increases of absolute and relative liver weights) at 108.5 mg/kg bw/d in the 2-year rat carcinogenicity study.

According to the CLP guidance, the guidance values to determine if a substance should be classified as STOT RE category 2 are: 100 mg/kg bw/d (in 90-day studies); 12.5 mg/kg bw/d (in 2-year studies); and 16.7 mg/kg bw/d (in 18-month studies). Thus, all the significant effects were reported at doses well-above those at which classification is warranted and therefore, **RAC** agrees with the DS **that S-methoprene does not fulfil the criteria for being classified as STOT-RE.**

4.8 Germ cell mutagenicity (Mutagenicity)

Table 28: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

Method	Results	Reference
In vitro		
Procaryote gene mutation		
Salmonella typhimurium: TA 1535, TA 1537, TA 98 with pKM101, TA 100 with pKM101 *Escherichia Coli* WP2 uvrA OECD 471	Negative +/- S9	Hernádi, D. (2002) CAR IIIA 6.6.1
Mammalian gene mutation		
None available		
Chromosomal aberrations		
Subline (KI) of Chinese hamster ovary cell line (CHO) OECD 473	Negative +/- S9	Béres, E. (2003) CAR IIIA 6.6.2
Subline (KI) of Chinese hamster ovary cell line (CHO) OECD 473	Negative +/- S9	Béres, E. (2002) CAR IIIA 6.6.3
DNA repair *in vitro*/cell transformation assay-mammalian		
None available		
In vivo		
Micronucleus assay		
None available		
Germ cell assays		
None available		

4.8.1 Non-human information

4.8.1.1 *In vitro* data

S-Methoprene did not induce gene mutations in bacterial cells *in vitro*. The *in vitro* chromosome aberration test in CHO cells revealed no evidence for clastogenic potential of S-Methoprene.

4.8.1.2 *In vivo* data

No *in vivo* data was available.

4.8.2 Human information

None available.

4.8.3 Other relevant information

None relevant.

4.8.4 Summary and discussion of mutagenicity

S-Methoprene was negative in both available *in vitro* studies.

4.8.5 Comparison with criteria

Not relevant.

4.8.6 Conclusions on classification and labelling

No classification.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for germ cell mutagenicity on the basis of the following results: i) one negative gene mutation assay in *Salmonella typhimurium* and in *Escherichia coli*; ii) two independent negative chromosomal aberration assays with Chinese hamster ovary (CHO) cells.

Comments received during public consultation

One MSCA argued that results from only two types of tests (bacterial gene mutation and chromosomal aberrations) is not sufficient and therefore the results for this hazard are not conclusive. In addition, the MSCA stated that this part of the CLH report was "very poorly detailed". The DS responded that the results of the available tests are conclusive, although not sufficient for proposing classification.

Assessment and comparison with the classification criteria

The table below summarises the available mutagenicity studies

METHOD	RESULTS	REMARKS
OECD TG 471 *Salmonella typhimurium*: TA 1535, TA 1537, TA 98 with pKM101, TA 100 with pKM101 *Escherichia Coli* WP2 uvrA	Negative +/- S9	Hernádi, 2002 Document IIIA 6.6.1 in S-methoprene CAR
OECD TG 473 CHO cell line	Negative +/- S9	Béres, 2003 Document IIIA 6.6.2 in S-methoprene CAR
OECD TG 473 CHO cell line	Negative +/- S9	Béres, 2002 Document IIIA 6.6.3 in S-methoprene CAR

The CLH report contained one study testing for gene mutation in *Salmonella typhimurium* (four different strains) and in *Escherichia coli,* performed according to OECD TG 471, and two independent studies for testing chromosomal aberrations in CHO cells performed according to OECD TG 473. All the studies yielded negative results with and without exogenous bioactivation.

RAC notes the absence of *in vivo* tests but recognises that all the available information points towards S-methoprene being negative for mutagenicity and therefore **RAC concludes that no classification is warranted** for germ cell mutagenicity.

4.9 Carcinogenicity

Table 29: Summary table of relevant carcinogenicity studies

Method	Results	Target organ/ principal effect at LOAEL	Reference
Rat, Charles River CD, Male/Female 50/sex/group Methoprene technical: 250, 1000 and 5000 ppm (equivalent to 10.9, 43.5 and 217.25 mg/kg bw/day) 104 weeks/daily	No treatment related tumours		Wazeter, F.X., Goldenthal, E.I., Geil, R.G., Benson, B.W., Keller, W.F. and Blanchard, G.L. (1975) CAR IIIA 6.4.1
Mouse, Charles River CD-1, Male/Female 50/sex/group Methoprene technical: 250, 1000 and 2500 ppm (equivalent to 0, 32.3, 130.4 and 325.9 mg/kg bw/day) 72- 78 weeks/daily	No treatment related tumours		Wazeter, F.X., Goldenthal, E.I., Geil, R.G., and Benson, B.W., (1975) CAR IIIA 6.4.1-2

Note: Methoprene is a terpenoid consisting of a racemic mixture of two enantiomers (R and S in a ratio of 1:1). The activity of Methoprene is restricted to the S enantiomer (S-Methoprene). Since S-Methoprene is the active isomer in the racemic mixture and as they are present in a 1:1 ratio it is possible to extrapolate the results obtained in the studies with Methoprene to S-Methoprene.

4.9.1 Non-human information

4.9.1.1 Carcinogenicity: oral

Study 1: Oral carcinogenicity study in the rat

Methoprene technical (86.9%) was administered at target dietary doses of 0, 250, 1000 and 5000 ppm daily over a period of 104 weeks to 4 groups of 50 Charles River CD rats/sex/group.

No changes in general behavior or appearance was deemed to be treatment related. Incidental findings noted such as occasional soft stool, slight alopecia, ocular or nasal porphyrin discharge and nodules (usually on the abdomen, thorax, or sides of the rats) were recorded in all groups.

Survival at 250 and 1000 ppm exceeded that of controls. 38% of males survived in the control group and at 5000 ppm. 52% and 48% of females survived in the control group and at 5000 ppm, respectively.

No treatment related changes were noted in bodyweight at any dose levels. Few statistically significant changes were reported between control and treated groups. However these were not deemed to be treatment related. Results of the statistical analysis are summarized in the following Table.

Table 30: Summary of body weights

Dosage level (ppm)	Number surviving/Number initiated	
	Male	Female
Control	19/50	26/50
250	27/50	30/50
1000	23/50	29/50
5000	19/50	24/50

Table 31: Summary of body weights

	Male (g)				Female (g)			
Week of Study	**0 ppm**	**250 ppm**	**1000 ppm**	**5000 ppm**	**0 ppm**	**250 ppm**	**1000 ppm**	**5000 ppm**
13	479	470	463	463	261	263	258	250
26	546	535	534	532	277	285	277	269
39	599	578	584	571	303	309	302	290
52	636	623	623	626	327	335	337	318
65	685	674	676	669	375	378	365	344[a]
78	675	662	656	677	377	394	371	344[a]
91	651	639	628	665	390	403	386	369
104	599	597	584	616	379	365	364	370

[a]Significantly different from control group mean, $p<0.05$

No treatment related changes were reported for food consumption at any dose level. No treatment related changes were recorded for haematology, biochemistry and urinalysis test. No ophthalmologic changes were reported during the study at any dose levels.

No treatment related gross pathologic lesions were noted at any dose levels. Few lesions such as mass skin, white yellow foci lung, abscess lung, enlarged spleen, hemorrhage erosion, ulcerations stomach and chronic nephritis were reported in all groups including the control. However they are commonly reported in rats of this age.

No treatment related effect on organ weight was reported. The absolute and relative weights of the liver were elevated in females at 5000 ppm dose level. Results are summarized in the following table.

Table 32: Summary of absolute (g) and relative (% body weight) organ weights

Group Sex	Body weight	Spleen		Liver		Adrenals		Kidneys		Testis		Ovaries		Heart		Thyroid/parathyroid		Brain		Pituitary	
	g	g	% x 10^2	g	%	g	% x 10^2	g	%	g	%	g	% x 10^2	g	%	g	% x 10^2	g	%	g	% x 10^2
Control																					
male	599	1.15	0.20	21.27	3.65	0.126	2.22	5.67	0.99	3.25	0.54	-	-	1.81	0.31	0.059	1.00	2.25	0.39	0.030	0.51
female	379	0.79	0.21	12.78	3.42	0.154	4.18	3.16	0.84	-	-	0.145	0.39	1.29	0.34	0.045	1.18	1.94	0.53	0.101	2.73
250 ppm																					
male	597	1.15	0.19	17.13[b]	2.90[a]	0.097[a]	1.69	4.70	0.81	3.70	0.62	-	-	1.93	0.33	0.047[a]	0.81	2.22	0.38	0.031	0.52
female	376	1.11	0.30	11.37	3.18	0.187	5.23	3.03	0.86	-	-	0.176	0.50[a]	1.36	0.38	0.048	1.35	1.96	0.55	0.151	4.69
1000 ppm																					
male	581	1.17	0.21	18.95	3.38	0.107	1.90	5.56	0.98	3.45	0.61	-	-	1.82	0.32	0.055	0.95	2.19	0.38	0.032	0.53
female	364	0.75	0.21	11.99	3.35	0.153	4.24	3.00	0.85	-	-	0.141	0.39	1.19	0.34	0.049	1.44	1.85	0.53	0.120	3.95
5000 ppm																					
male	616	1.10	0.18	21.10	3.45	0.089[a]	1.49[a]	5.47	0.91	3.23	0.53	-	-	1.73	0.29	0.063	1.05	2.07[b]	0.34	0.027	0.45
female	370	0.65[a]	0.18	15.41	4.13[b]	0.143	4.08	3.14	0.86	-	-	0.149	0.43	1.25	0.34	0.051	1.47	1.86	0.52	0.110	3.22

Group mean relative organ weights shown in this table were calculated by averaging the individually calculated relative organ weights

[a]Significantly different from control group mean, $p<0.05$

[b]Significantly different from control group mean, $p<0.01$

No treatment related histopathologic lesions were noted at any dose levels. Few lesions such as chronic nephritis and adenoma pituitary were noted in control and high dose groups. However they are commonly reported in rats of this age. Histopathological evaluation showed an increased incidence of hepatic lesions, such as bile-duct proliferation and portal lymphocyte infiltration in males at 5000ppm dose level.

No treatment related tumours were reported. There were no statistically significant changes at any dose levels.

Based on the increased incidence of hepatic lesions such as bile-duct proliferation and portal lymphocyte infiltration in males at 5000 ppm dose level and the increased absolute and relative weights of the liver in females at 5000 ppm dose level, the NOEL was established to be 1000 ppm (equivalent to 43.45 mg/kg bw/day). Methoprene is concluded to be non-carcinogenic at dose levels up to 5000 ppm (equivalent to 217 mg/kg bw/day).

Methoprene is a terpenoid consisting of a racemic mixture of two enantiomers (R and S in a ratio of 1:1). The activity of Methoprene as a juvenile hormone is restricted to the S enantiomer (S-Methoprene). Since S-Methoprene is the active isomer in the racemic mixture and as they are present in a 1:1 ratio it is possible to extrapolate the results obtained in the studies with Methoprene to S-Methoprene. Therefore, the observed NOAEL in this study has been revised and a NOAEL for S-Methoprene is proposed:

- NOAEL for S-Methoprene = **21.72 mg/kg bw/day**

In general the level of reporting in this study is poor. There is no reference to findings at the high dose which may have been attributable to treatment, e.g., relative liver weight increase in high dose females; an apparent increase in liver histopathological findings in high dose animals (portal lymphocytic infiltration, bile duct proliferation, small groups of vacuolated hepatocytes).

Survival was lower than average for some male groups including controls (19/50, 27/50, 23/50, 19/50, i.e., 38%, 54%, 46%, 38% respectively). There does not seem to be a relationship with treatment. In order for a test to be considered negative for carcinogenicity, survival should be no less than 50%. Survival was less that 50% in the male controls, 1000, and 5000ppm groups.

It is noted that there was a very high incidence of lung disease in all groups.

Some data entries were missing from the Table 24 (no thymus input for 30/43 animals).

There was no discussion of these results in the light of the findings of the 90-day rat study (A6.4.1) where a statistically significant ($p<0.01$) and treatment-related increase in absolute and relative liver weight was recorded from 200 mg/kg bw/day (lowest dose). Relative kidney weight was also significantly elevated in both sexes.

Study 2: Oral carcinogenicity study in the mouse

Methoprene technical (86.9 and 87.5%) was administered at target dietary doses of 0, 250, 1000 and 2500 ppm daily over a period of 72 weeks to 4 groups of 50 Charles River CD-1 mice/sex/group. Duration of treatment: 72 weeks for females treated at 1000 ppm and 78 weeks for all other groups. At study termination, all surviving mice were sacrificed, necropsied and fixed *in toto* in buffered neutral 10% formalin. Mice that died during the study were also necropsied and their tissues were saved if autolytic changes were not advanced.

No changes were observed in the mouse carcinogenicity study considered to be treatment related were seen in the general behavior and appearance of the animals. Females of the 1000ppm group were sacrificed at 72 weeks. No reason is given. It can be assumed that this was because survival had fallen to 20/50 (40%). The OECD criteria for a negative (carcinogenicity) test include the stipulation

that survival of all groups should be no less than 50%. Survival was lower than 50% in all treated groups of this study. Mortality rates are described in the following table.

Table 33: Summary of mortality results

Dosage level (ppm)	Number surviving/number initiated	
	Male	Female
Control 1	32/50	25/50
250	28/50	22/50
1000	27/50	20/50[a]
2500	30/50	24/50

[a] prior to sacrifice, week 72

All animals gained weight throughout the study. Increases in body weight were similar for control and treated mice. No changes in food consumption were reported in both sexes at any dose level.

It was noted that the treatment-related incidence of liver pathology (dark brown, finely granular pigment were observed in the cytoplasm of liver parenchymal cells of most male and female mice sacrificed at study termination at 2500 ppm. Many mice also displayed focal accumulations of macrophages with brownish, foamy cytoplasm in their livers. These changes were associated with small necrotic foci and mononuclear inflammatory cells. Less frequently, intracytoplasmic brown pigment was detected in Kupffer cells) was greater and more marked in the 2500-ppm females and in the 10 females from the 1000 ppm survivors. These pigmentary changes were not noted in mice at 250 ppm.

The intracytoplasmic pigment in the hepatocytes was reported to be PAS negative, negative for oil red-o stain and negative for iron. The macrophages stained positive. The composition of the treatment-related pigment accumulation is not known, therefore. It is likely to reflect some toxicity related endpoint and leads to the conclusion that following long-term exposure in the diet, methoprene resulted in probably toxicity-related liver pathology in the mouse, with females being more sensitive. The NOEL for this effect was 250 ppm.

Based on the presence of focal accumulations of macrophages with brownish foamy cytoplasm in the liver, often associated with small necrotic foci and mononuclear inflammatory cells, the NOEL for Methoprene technical under the conditions of this study was established as 1000 ppm (equivalent to 130.8 mg/kg bw/day).

No compound related tumourigenic effect was observed at any dose level. Incidence of the more commonly occurring tumours was similar between the control and the treated groups. There were no statistical differences reported. Furthermore overall tumour incidence was similar between this study and other 18-month studies conducted in this laboratory in this strain of mouse

Methoprene is not concluded to be carcinogenic at dose levels up to 2500 ppm (equivalent to 327 mg/kg bw/day).

Methoprene is a terpenoid consisting of a racemic mixture of two enantiomers (R and S in a ratio of 1:1). The activity of Methoprene as a juvenile hormone is restricted to the S enantiomer (S-Methoprene). Since S-Methoprene is the active isomer in the racemic mixture and as they are present in a 1:1 ratio it is possible to extrapolate the results obtained in the studies with Methoprene

to S-Methoprene. Therefore, the observed NOEL in this study has been revised and a NOEL for S-Methoprene is proposed:

4.9.1.2 Carcinogenicity: inhalation

No data

4.9.1.3 Carcinogenicity: dermal

No data

4.9.2 Human information

No data

4.9.3 Other relevant information

4.9.4 Summary and discussion of carcinogenicity

Technical Methoprene (86.9%) was administered to rats in the diet, in a combined carcinogenicity and chronic toxicity study, at nominal concentrations of up to 5000 ppm (equivalent to 108.6 mg/kg bw/day S-Methoprene) for 24 months. There was no evidence of carcinogenicity in this study. Similarly, there was no evidence of carcinogenicity in the mouse following 72-78 weeks of dietary administration of technical Methoprene (86.9% and 87.5%) at nominal concentrations of up to 2500 ppm (equivalent to 163.5 mg/kg bw/day S-Methoprene).

4.9.5 Comparison with criteria

The CLP criteria for classification as a category 1 Carcinogen are as follows:

"Known or presumed human carcinogens. A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as: Category 1A: Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B: Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations. Such evidence may be derived from:

— human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or

— animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen).

In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals".

The CLP criteria for classification as a category 2 Carcinogen are as follows:

"*Substances are classified as a category 2 Carcinogen when evidence is obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B,*

based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from ***limited evidence*** *of carcinogenicity in animal studies."*

There are two studies, one rat, and one mouse available for S-Methoprene. Carcinogenic potential is not demonstrated in either. Based on the finding of the aforementioned studies and the lack of any other data suggesting carcinogenicity no carcinogenicity classification is required.

4.9.6 Conclusions on classification and labelling

Based upon the findings of the submitted studies classification of S-Methoprene for carcinogenicity is not required.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of S-methoprene for carcinogenicity on the basis of two studies showing no evidence of carcinogenicity in rats exposed for 2 years at up to 108.6 mg/kg bw/d and in mice exposed during 18 months at up to 163.5 mg/kg bw/d.

Comments received during public consultation

One MSCA queried whether the studies were performed according to OECD guidelines or similar. The DS responded that both studies were carried out prior to the availability of US EPA and OECD guidelines. However, both studies were compared with the requirements of OECD TG 453 and 451 for evaluation purposes. The DS also stated that the deviations from the OECD guidelines were documented by both the Applicant and the Competent Authority and were considered during the evaluation of these studies. It was noted in the DAR (Document IIIA) that the studies were considered reliable with restrictions despite the deficiencies relative to the TG.

Assessment and comparison with the classification criteria

The CLH report contains information about carcinogenicity coming from two different studies.

Oral carcinogenicity study in rat (Wazeter et al., 1975, Document IIIA 6.4.1 in S-methoprene CAR)

Methoprene technical product (96%) was administered at target dietary doses of 0, 5.45, 21.7 and 108.5 mg/kg bw/d of S-methoprene over a period of 104 weeks to 4 groups of 50 Charles River CD rats/sex/group. No changes in general behaviour or appearance were deemed to be treatment related. Incidental findings noted such as occasional soft stool, slight alopecia, ocular or nasal porphyrin discharge and nodules (usually on the abdomen, thorax, or sides of the rats) were recorded in all groups.

No treatment related tumours were reported in this study. However, no information on any tumours that might have been observed (apart from a reference to pituitary adenomas having been seen in high dose and control groups) was provided in the CLH report (or the CAR) and therefore RAC notes that an independent verification was not possible. There were no statistically significant changes at any dose level.

No treatment related changes were noted in bodyweight at any dose level for male rats. Some statistically significant changes in female bodyweight were reported between the control and high dose group only in week 65 and 78 of the study. However, these were not deemed to be treatment related.

No treatment related changes were reported for food consumption at any dose level. No treatment related changes were recorded in haematology, biochemistry and urinalysis tests. No ophthalmologic changes were reported during the study at any dose levels.
No treatment related gross pathologic lesions were noted at any dose levels. Few lesions such as mass skin, white yellow foci lung, abscess lung, enlarged spleen, haemorrhage erosion, ulcerations of the stomach and chronic nephritis were reported in all groups including the control. However, they are commonly reported in rats of this age.

No treatment related effects on organ weights were reported. The absolute and relative weights of the liver were elevated in females at the 108.5 mg/kg bw/day dose level.

No treatment related histopathologic lesions were noted at any dose levels. Some lesions such as chronic nephritis and adenoma of the pituitary were noted in control and high dose groups, but incidences were not reported in the CLH report. However, they are commonly reported in rats of this age. Histopathological evaluation showed an increased incidence in hepatic lesions, such as bile-duct proliferation and portal lymphocyte infiltration in males at the 108.5 mg/kg bw/d dose level.

The survival rates in the control, 21.7 and 108.5 mg/kg bw/d groups were 38%, 46% and 38%, respectively, the survival rate being greater than 50% only for the 5.45 mg/kg bw/d group.

Oral carcinogenicity study in mouse (Wazeter et al., 1975, Document IIIA 6.4.2 in S-methoprene CAR)

Methoprene technical product (86.9 and 87.5%) was administered at target dietary doses of 0, 16.35, 65.4 and 163.5 mg/kg bw/d of S-methoprene to 4 groups of 50 Charles River CD-1 mice/sex/group. The duration of the treatment was 72 weeks for females treated at 65.4 mg/kg bw/d and 78 weeks for all other groups. At study termination, all surviving mice were sacrificed and necropsied. Mice that died during the study were also necropsied.

No compound related tumourigenic effect was observed at any dose level. The incidence of the more commonly occurring tumours was similar between the control and the treated groups. There were no statistically significant differences reported. Furthermore, the overall tumour incidence was similar between this study and other 18-month studies conducted in this laboratory in this strain of mouse. However, no information on the specific tumours observed in this study or their incidences was provided in the CLH report (or the CAR) and therefore RAC notes that an independent verification was not possible.

No changes were observed that were considered to be treatmentrelated in the general behaviour and appearance of the animals. Survival rates ranged between 54% and 64% for males and between 40% and 50% for females, but no relationship between dose and survival could be established.

All animals gained weight throughout the study. Increases in body weight were similar for control and treated mice. No changes in food consumption were reported in either sex at any dose level.

It was noted that treatment related liver pathology (dark brown, finely granular pigment) was observed in the cytoplasm of liver parenchymal cells of most male and female mice sacrificed at study termination at the dose of 163.5 mg/kg bw/d. Many mice also displayed focal accumulations of macrophages with brownish, foamy cytoplasm in their livers. These changes were associated with small necrotic foci and mononuclear inflammatory cells. Intracytoplasmic brown pigment was detected in Kupffer cells in the 163.5 mg/kg bw/d females and in the 10 female survivors from the 65.4 mg/kg bw/d group. These pigmentary changes were not noted in mice at 16.35 mg/kg bw/d.

Conclusion

The studies in the rat and mouse failed to show any carcinogenic potential for S-methoprene. RAC notes that the maximum tolerable dose may not have been reached in either study because the general toxicity was mild even at the highest dose (no decreases of body weight were noted and the liver impairments did not appear to cause haematological alterations). In addition, the available information does not demonstrate any mutagenic potential of S-methoprene. Thus, the available information does not indicate that S-methoprene should be considered as a suspected human carcinogen (category 2) and as a consequence RAC agrees with the DS that **no classification for S-methoprene for carcinogenicity is warranted**.

Nevertheless, RAC highlights that a more detailed description of the carcinogenic lesions was necessary because with this level of reporting RAC was unable to independently verify whether or not the tumours observed in either study were treatment related.

4.10 Toxicity for reproduction

Table 34: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
*Oral, Rat, Charles River albino female rats, 19-22/group. Day 6-15 of gestation. Methoprene technical: 500 and 1000 mg/kg/day.	Maternal toxicity NOAEL Methoprene technical: 1000 mg/kg bw/day. S-Methoprene: 500 mg/kg/day		Haley, S. (1972)

The study was carried out before the availability of US EPA and OECD guidelines.	Bifurcated sternum, cleft sternum and sternal asymmetry at 1000 mg/kg bw/day. Teratogenicity Embryotoxicity NOAEL: Methoprene 500 mg/kg bw/day and S-Methoprene 250 mg/kg/day.		
OECD Guideline 414, Oral, Rabbit, New Zealand White , Female, 17-25/group. Day 6-27 of gestation. S-Methoprene technical: 25, 100 and 1000 mg/kg/day	Maternal toxicity NOAEL S-Methoprene: 100 mg/kg bw/day. NOAEL Growth retarded foetuses, treatment related maternal death, abortions and vaginal bleeding at 1000 mg/kg bw/day. Teratogenicity Embryotoxicity : 100 mg/kg bw/day		Kolep Csete, K. (2008) CAR IIIA 6.8.1-1
OECD Guideline 414, Oral, Rat , Hsd. Brl. Han: WIST Rats, Female, 22-26/group. Day 5-19 of gestation. S-Methoprene technical: 60, 250 and 1000 mg/kg/day	Maternal toxicity NOAEL S-Methoprene: 250 mg/kg bw/day. NOAEL ↓food consumption and mean body weight gain. ↑post-implantation loss. Teratogenicity Embryotoxicity : 250 mg/kg bw/day		Kolep Csete, K. (2009) CAR IIIA 6.8.1-2

* The rat gavage study of *S. Haley* (1972) was considered invalid because of serious methodological and reporting inadequacies and was not considered in the assessment.

Note: Methoprene is a terpenoid consisting of a racemic mixture of two enantiomers (R and S in a ratio of 1:1). The activity of Methoprene is restricted to the S enantiomer (S-Methoprene). Since S-Methoprene is the active isomer in the racemic mixture and as they are present in a 1:1 ratio it is possible to extrapolate the results obtained in the studies with Methoprene to S-Methoprene.

4.10.1 Effects on fertility

4.10.1.1 Non-human information

Study 1: Teratogenic study with Altosid technical in albino rats. Haley, S., (1972) - CAR IIIA 6.8.1(1)

Conclusion: The rat gavage study of *S. Haley (1972)* was considered invalid because of serious methodological and reporting inadequacies and therefore was not used in the risk assessment.

Study 2: Teratology study of test item S-Methoprene technical in rabbits. Kolep, C.K. (2008) - CAR IIIA 6.8.1(2)

Four groups of 25 inseminated female New Zealand albino white rabbits were dosed, once daily between days 6 to 27 of presumed gestation, by the oral route with S-Methoprene at dose levels of 0, 25,100 and 1000 mg/kg bw/day. On gestation day 28, euthanasia of the animals was carried out. The internal organs were examined macroscopically and all changes were recorded. The number of corpora lutea in each ovary, the number of implantation sites in each uterine horn, the number of live foetuses, early and late embryonic deaths and dead foetuses were determined. The uterus with cervix and the left ovary were removed and weighed. Live foetuses were individually weighed. The crown

rump length of the foetuses and the litter mean was calculated. All foetuses were examined externally and viscerally and the sex of the foetuses was determined.

Maternal effects:

Body weight

Clear maternal toxicity was evidenced by marked adverse effects on mean body weight, body weight gains and corrected body weights and some clinical signs observed at the top dose. Treatment related changes in mean maternal body weight, body weight gain and corrected body weight were seen 1000 mg/kg bw/day dams. A treatment-related and statistically significant ($p < 0.01$) reduction in bodyweight from was seen from day 18 in the 1000 mg/kg bw/day dose group. Body weight gain was statistically significantly ($p<0.01$) reduced throughout the treatment period. The gravid uterine weight ($p<0.05$), corrected body weight ($p<0.01$) and corrected body weight gain ($p<0.01$) were also statistically significantly lower for the 1000 mg/kg bw/day dose group.

Table 35: Mean maternal body weights (g)

Treatment group	Gestation day (TIME)					
	0	6	12	18	24	28
Control	4009.7 ±236.18	4221.1 ±348.34	4258.3 ±418.72	4433.4 ±387.19	4529.4 ±371.65	4600.0 ±345.08
25 mg/kg/day	3990.0 ±210.69	4242.0 ±223.67	4323.4 ±209.57	4472.5 ±250.09	4530.3 ±271.85	4543.9 ±248.51
100 mg/kg/day	4053.9 ±198.08	4309.2 ±212.84	4408.3 ±216.45	4560.3 ±246.25	4634.3 ±279.61	4659.2 ±302.37
1000 mg/kg/day	4022.8 ±194.26	4267.8 ±207.81	4146.4 ±252.90	4104.2** ±369.64	4097.2** ±416.93	4126.4** ±503.51

*= Statistically significant at $p < 0.05$
**= Statistically significant at $p <0.01$

Table 36: **Mean maternal body weights gain (g)**

Treatment group	Gestation day (TIME)					
	0-6	6-12	12-18	18-24	24-28	0-28
Control	211.4 ±171.16	37.2 ±146.93	175.1 ±114.72	96.1 ±94.52	70.6 ±139.65	590.3 ±217.08
25 mg/kg/day	252.0 ±79.04	81.3 ±116.13	149.2 ±126.40	57.7 ±125.07	13.6 ±155.58	553.9 ±180.43
100 mg/kg/day	255.3 ±119.91	99.1 ±112.55	152.0 ±95.30	74.0 ±84.94	25.0 ±128.05	605.4 ±233.68
1000 mg/kg/day	245.0 ±69.65	-111.3** ±132.98	-42.2** ±222.91	-53.7* ±215.57	-14.8 ±237.31	141.2** ±412.06

*= Statistically significant at $p < 0.05$
**= Statistically significant at $p <0.01$

Mortality

Three does died during the study, two in the control and one at 1000 mg/kg bw/day group. The top dose doe died following abortion and this was considered treatment-related. The two control animals were considered to have died of internal bacterial infection, therefore these deaths were unrelated to treatment.

Abortions and clinical signs
One animal aborted and was sacrificed in the low dose group on gestation day 21. Six animals aborted in the high dose group, (one died following abortion and five were sacrificed after abortion). Except for the doe, which died following abortion on gestation day 17, all the abortions in the high dose group were after the organogenetic period (from gestation day 18) and these rabbits had marked weight loss by the end of the first week of the treatment period. Vaginal bleeding was reported in one control animal, one animal dosed at 25 mg/kg bw/day and two animals dosed at 1000 mg/kg bw/day. One doe at 1000 mg/kg bw/day had bloody urine and blood was found in the cage. A high incidence in the late embryonic death was reported in this doe. Total intrauterine mortality was also noted in the doe that showed vaginal bleeding. The incidences of abortions and vaginal bleeding in the other treatment groups were within the normal range.

Clinical signs included diarrhoea in one animal at 25 mg/kg bw/day and soft faeces in 3, 4 and 6 animals in the 25, 100 and 1000 mg/kg bw/day dose groups, respectively. This was accompanied by reduced body weight in the 1000 mg/kg bw/day dose group. In addition, reduced activity noted in five rabbits in the high dose group was considered related to treatment.

Necropsy and pathology
No treatment related changes were reported at necropsy. One animal that died in the 1000 mg/kg bw/day dose group following abortion showed dark reddish mottled lungs, bloody discharge in the thoracic cavity and nutmeg like patterned lungs. These findings were considered related to abortion and physiological stress. Dead animals in the control group displayed necropsy findings such as blood around nasal orifice, dark reddish mottled lungs and inflammation exudates in the thoracic cavity. These deaths were not treatment-related and were as a result of inter-current or accidental bacterial infection. The maternal reproductive parameters, overall pregnancy rate, number of corpora lutea, number of implantations were similar in all groups. There were no treatment related changes at histopathological examination.

In summary, there was evidence of maternal toxicity characterized by a significantly reduced mean body weights, body weight gains and corrected body weights at the high dose. Increased instances of treatment-related abortions and vaginal bleeding in the 1000 mg/kg/bw/day dose group were considered treatment-related also. The overly severe effects on the maternal animal do not allow discrimination between the endpoints of maternal and developmental toxicity at the top dose, if such exists with this substance.

Embryo and foetal effects:

The number of viable foetuses in the treated groups was comparable to controls. The number of female foetuses in the 1000 mg/kg bw/day dose group was statistically significantly higher than males (60 v's 40%). However, this incidence was not deemed to be treatment related.

Table 37: Intrauterine mortality, viable foetuses and their sex distribution

Group		Autopsy findings (Mean/Female)			
		Control	25 mg/kg bw/day	100 mg/kg bw/day	1000 mg/kg bw/day
No. Foetuses examined		19	22	21	18

Corpora Lutea	**Mean**	11.5	10.7	11.1	11.1
	SD	3.47	2.16	2.10	2.74
Pre-implantation sites	**Mean**	11.2	18.3	14.9	13.1
	SD	11.83	17.49	19.49	14.97
Implantation	**Mean**	10.4	8.7	9.5	9.8
	SD	3.47	2.41	2.69	3.30
Early Embryonic Death %	**Mean**	11.5	1.1	2.1	10.9
	SD	25.10	3.85	4.55	23.62
Late Embryonic Death %	**Mean**	4.6	3.3	3.5	7.4
	SD	5.96	5.60	5.90	16.79
Dead Foetuses %	**Mean**	1.6	0.5	5.1	2.0
	SD	3.19	2.35	21.80	6.07
Post-implantation Loss %	**Mean**	17.7	4.9	10.8	20.4
	SD	24.5	7.00	21.82	28.25
Total intrauterine mortality %	**Mean**	27.3	22.6	24.6	29.6
	SD	24.54	16.68	26.62	27.01
Viable Foetuses	**Mean**	8.5	8.2	8.5	7.7
	SD	3.45	2.02	3.30	2.97
Male Foetuses % (DN)	**Mean**	57.1	47.0	53.7	40.2*
	SD	18.58	20.69	16.19	17.82
Female Foetuses % (DN)	**Mean**	43.0	53.2	46.5	60.0*
	SD	18.56	20.72	16.05	17.85

*= $p < 0.05$

DN= Duncan's Multiple Range Test

Table 38: Intrauterine mortality, viable foetuses and their sex distribution

Group		**Autopsy findings (Mean/Female)**			
		Control	**25 mg/kg bw/day**	**100 mg/kg bw/day**	**1000 mg/kg bw/day**
No. Foetuses examined		19	22	21	18
Corpora Lutea	**Sum**	218	236	234	200
Pre-implantation sites	**Sum**	24	45*	37	24
(Data compared to no. of corpora lutea)	**%**	11	19	16	12

Implantation	**Sum**	198	192	199	176
Early Embryonic Death %	**Sum**	23	3**	4**	16
(Data compared to no. of implantations)	**%**	12	2	2	9
Late Embryonic Death %	**Sum**	10	7	7	17
(Data compared to no. of implantations)	**%**	5	4	4	10
Dead Foetuses %	**Sum**	4	1	9	4
(Data compared to no. of implantations)	**%**	2	1	5	2
Post-implantation Loss %	**Sum**	37	11**	20*	37
(Data compared to no. of implantations)	**%**	19	6	6	21
Total intrauterine mortality %	**Sum**	61	56	57	61
(Data compared to no. of corpora lutea)	**%**	28	24	24	31
Viable Foetuses	**Sum**	161	181	179	139
Male Foetuses	**Sum**	89	86	96	55**
(Data compared to no. of viable foetuses)	**%**	55	48	54	40
Female Foetuses	**Sum**	72	95	83	84**
(Data compared to no. of viable foetuses)	**%**	45	52	46	60

Remarks:
*= Statistically significant at p<0.05 Chi Square
**= Statistically significant at p<0.01 Chi Square

External findings

A biologically and statistically significantly lower foetal body weight (p<0.05) and crown-rump length averages (p<0.01) were found in the 1000 mg/kg bw/day dose group. Significant maternal toxicity was demonstrated at this dose level. The number of foetuses with abnormalities was significantly higher (p<0.01) in the 1000 mg/kg bw/day dose group, The overall incidence of variations was statistically significantly higher in the 1000 mg/kg bw/day dose group when compared to the control. This difference was due to the higher incidence of growth retarded fetuses (body weight and crown-rump length evaluated as variations). A number of external variations (misaligned palatine rugae, protruding tongue and bent tail) were reported in the control and treated groups, without dose relationship.

Table 39: Summary of gravid uterine weight, corrected body weight and body weight gain of does

		Dose Groups				
		Control	**25 mg/kg**	**100 mg/kg**	**1000 mg/kg**	
gravid uterine Weight (g)	Mean	542.7	491.3	525.3	437.6	
	SD	163.81	97.80	125.53	84.08	
	n	18	22	20	17*	U

corrected body weight (g)	Mean SD n	4092.1 285.79 18	4052.6 275.83 22	4152.1 235.31 20	3784.3 271.48 17**	 DN
corrected body weight gain (g)	Mean SD n	66.1 188.89 18	62.6 178.09 22	88.7 182.73 20	-219.1 227.36 17**	 DN

Remarks:
*= Statistically significant at p<0.05
**= Statistically significant at p<0.01
U=Mann-Whitney U-Test Versus Control
DN= Duncan's Multiple Range Test

Visceral examination.
The incidence of rudimentary lung-lobe was significantly higher in the 1000 mg/kg bw/day dose group. The incidence of absent lung-lobe was significantly higher in the 25 mg/kg bw/day and in the 1000 mg/kg bw/day dose group. However, these values were within the historical control range. None of these differences were considered treatment related.

Skeletal examination.
Significantly higher incidences of enlarged anterior fontanelle and un-ossified proximal phalanges of pellex (bilateral) were noted. These incidences resulted in a significant increase in the incidence of foetuses with abnormalities and with variations in the 1000 mg/kg bw/day dose group. These changes were correlated to the intrauterine foetal growth retardation and were deemed to be treatment related.

The average incidence of skeletal malformations was lower in the treated groups than in the control groups. A number of skeletal malformations were recorded in all study groups including controls: (fused sternal bodies, wide sternal bodies, branched and /or fused rib cartilages or branched rib). However, the skeletal malformations in all groups represented a normal background incidence and a statistical evaluation of skeletal parameters showed that all parameters were within the expected range.

Table 40: Results of external, visceral and skeletal examinations

Dose Groups					
		Control	**25 mg/kg**	**100 mg/kg**	**1000 mg/kg**
Litters examined	N	18	22	20	17
EXTERNAL EXAMINATION					
Foetuses examined	N	161	181	179	139
Foetuses with abnormalities	N %	13 8	8 4	16 9	29** 21
Variation	N %	12 7	5* 3	13 7	27** 19
Malformation	N %	1 1	3 2	3 2	2 1
Retarded in body weight	N %	7 4	4 2	11 6	15* 11
Retarded in crown-rump weight	N %	6 4	4 2	11 6	22** 16
VISCERAL EXAMINATION					
Foetuses examined	N	161	181	179	139

Foetuses with abnormalities	N %	31 19	37 20	33 18	39 28
Variation	N %	28 17	32 18	31 17	37 27
Malformation	N %	3 2	5 3	2 1	2 1
SKELETAL EXAMINATION					
Foetuses examined	N	152	181	171	139
Foetuses with abnormalities	N %	19 13	18 10	19 11	30* 22
Variation	N %	11 7	14 8	14 8	26** 19
Malformation	N %	8 5	4 2	5 3	4 3
NO. of FOETUSES WITH VARIATION					
	N %	43 27	48 27	48 27	68** 49
No. FOETUSES WITH MALFORMATION					
	N %	10 6	8 4	9 5	7 5

Remarks:
*= Statistically significant at p<0.05 Chi Square
**= Statistically significant at p<0.01 Chi Square

Conclusion:
Severe maternal toxicity (death, weight loss) was accompanied by significant foetolethality (abortions) and foetotoxicity (runts and retarded ossification) at the high dose of 1000 mg/kg bw/day. However, an NOEL was established for maternal and foetal toxicity at 100-mg/kg bw/day.

Ultimately, the dosing in the rabbit developmental study is considered inadequate. The top dose is considered to be inappropriately high and the mid-range dose provides an NOEL value. However, this NOEL value of 100mg/kg bw/day is used as the NOAEL value and is used to establish a systemic AEL acute reference value even though it may be a more conservative value than what may have been achieved if the study dosing was more appropriately considered.

Study 3: Teratology study of test item S-Methoprene technical in rats. Kolep, C.K. (2009) - CAR IIIA 6.8.1(2)

In a new study, the teratogenicity of S-Methoprene Technical was investigated by oral administration to 4 groups of pregnant female rats at the following concentrations: 0, 60, 250 and 1000 mg/kg bw/day followed by full examination of the dams and the foetuses. The number of sperm positive females in the study was 97. None of the females displayed clinical signs and all females survived until necropsy on gestation day 20.

Maternal Effects:

1000 mg/kg bw/day dose group: There was a statistically significant reduction in body weight gain between gestational days 17 to 20 (p<0.01) and 0 to 20 (p<0.05) compared to the vehicle control level. Corrected body weight was lower in this treatment group than in other experimental groups. However, this was not statistically significant due to the high standard deviation, but was considered biologically relevant. Food consumption of the dams was statistically significantly reduced (p<0.05) during the whole treatment period. This was considered to be treatment related. Mottled reddish

lungs were observed at necropsy for four dams in the 60 and 1000 mg/kg bw/day groups. This finding was attributed to euthanasia and was not considered to be treatment related.

250 mg/kg bw/day dose group: There were four non-pregnant animals in this treatment group. At termination on gestation day 20, there were 18 dams with live fetuses and more than five implantations. There were no changes observed in body weight, body weight gain or food consumption. At necropsy, there were no treatment related effects noted.

60 mg/kg bw/day dose group: There were two non-pregnant animals in this treatment group. At termination on gestation day 20, there were 19 dams with live foetuses and more than five implantations. In the 60 mg/kg bw/day treatment group, on necropsy, reddish mottled lungs were observed in four dams in this treatment group. This effect was attributed to euthanasia and was not considered to be treatment related. There were no changes observed in body weight, body weight gain or food consumption. At necropsy, there were no treatment related effects noted.

Table 41: Mean maternal body weights and body weight changes in the teratogenicity study with S-Methoprene Technical

Day of gestation	Dosage level (mg/kg bw/day)			
	Group mean maternal body weights (g)			
	0 (Control)	60	250	1000
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0	205.7 ± 16.10	198.4 ± 15.30	196.8 ± 11.49	201.8 ± 20.99
5	224.7± 15.74	219.9 ± 16.72	217.4 ± 11.89	221.9 ± 20.36
11	245.5 ± 15.49	243.1 ± 18.54	240.0 ± 16.86	239.8 ± 21.94
17	280.5 ± 20.93	278.1 ± 21.58	275.8 ± 19.93	273.3 ± 28.77
20	313.2 ± 22.56	309.1 ± 22.47	303.3 ± 25.23	291.7 ± 35.41
Days of gestation	Group mean maternal body weight gain (g)			
0 to 5	19.0 ± 6.01	21.5 ± 6.00	20.7 ± 5.12	20.1 ± 6.81
5 to 11	20.8 ± 5.10	23.2 ± 5.73	22.6 ± 7.33	17.8 ± 6.50
11 to 17	34.6 ± 10.44	34.9 ± 7.04	35.8 ± 10.06	33.5 ± 10.91
17 to 20	33.1 ± 9.81	31.1 ± 4.39	27.6 ± 14.73	18.4** ± 17.98
0 to 20	107.5 ± 16.39	110.7 ± 17.46	106.6 ± 21.40	89.9* ± 28.36

Significantly different from control:
* p<0.05
** p<0.01

Table 42: Mean maternal food consumption in the teratogenicity study with S-Methoprene Technical

Day of gestation	Dosage level (mg/kg bw/day)			
	Food consumption (g)			
	0 (Control)	60	250	1000
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0 to 5	20.7 ± 3.15	21.1 ± 2.79	20.9 ± 1.21	20.5 ± 3.46
5 to 11	20.0 ± 1.25	20.5 ± 1.72	20.9 ± 1.70	18.1* ± 3.06
11 to 17	21.4 ± 1.15	21.6 ± 1.94	21.9 ± 1.74	19.5* ± 2.48
17 to 20	22.6 ± 1.99	23.3 ± 2.48	21.9 ± 2.16	20.6* ± 2.35

Significantly different from control:
* p<0.05

Embryo and foetal effects:

Foetal body weight was not influenced by treatment with the test substance.

1000 mg/kg bw/day dose group: There was a statistically significant reduction in post-implantation loss at this dose level. There was no treatment related effect observed on either the type or incidence of malformations or variations during skeletal examination of the foetuses.

250 mg/kg bw/day dose group: Pre-implantation loss increased statistically significantly (13%, $p<0.05$) in this treatment group, however, it was below the historical control level (20%). No external or visceral malformations were noted in the 250 mg/kg bw/day treatment group. One foetus in the 250 mg/kg bw/day group displayed signs of vertebral abnormalities, having lumbar vertebrae malformations. However, this was not considered to be treatment related.

60 mg/kg bw/day dose group: In the 60 mg/kg mw/day treatment group, incomplete ossification of the skull was noted in three foetuses, while marked wavy ribs with or without complete ossification was found in six foetuses in the control and two in this treatment group. Vertebral abnormalities were found in three foetuses in the control and 60 mg/kg bw/day treatment group. There were no visceral malformations in this treatment group. None of the skeletal malformations were increased significantly in the test substance treatment groups

No treatment related foetal external abnormalities or visceral examinations were observed in the foetal litter. None of the skeletal malformations were increased significantly in the treatment groups.

Table 43: Pregnancy data in the teratogenicity study with S-Methoprene Technical

Dose groups	Dosage level (mg/kg bw/day)			
	0 (Control)	**60**	**250**	**1000**
No. of sperm positive females	26	22	22	27
Percentage of pregnant females	89 %	91 %	82 %	70 %
No. of non-pregnant females	3	2	4	8
No. of pregnant females with no implantation but corpora lutea	0	0	0	1
No. of dams with total intrauterine death	0	0	0	1
No. of pregnant females with viable foetuses, but with less than or equal to 5 implantations	0	1	0	1
Percentage of evaluated dams with malformed foetuses	39 %	37 %	11 %	25 %

Conclusion:
Maternal toxicity was evidenced at the high dose by a statistically significant reduction in food consumption and mean weight gain. There was also a statistically significant reduction in post-

implantation loss at this dose level. A NOEL of 250 mg/kg/day was established for both maternal and developmental toxicity.

4.10.1.2 Human information

No data.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

Table 44: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Rat, Long-Evans, Male and female, 20 animals /sex/ dose. Over a three generation period **Methoprene technical**: 500 and 2500 ppm equivalent to 0, 16.3 and 261.6 mg/kg bw/day.	Overall, minimal and insufficient parental toxicity was demonstrated in this study. The only effects were slight reduction in mean pup weights seen at day 21 of lactation in the F2 generation and throughout lactation in the F3 generation.	The study was carried out before the availability of US EPA and OECD guidelines. The dosing parameters for testing the substance were not extended sufficiently to produce the required parental toxicity and as a consequence this substance was not assessed to its full extent	Killeen, J.C., Rapp, W.R. (1974) CAR IIIA 6.8.2

Note: Methoprene is a terpenoid consisting of a racemic mixture of two enantiomers (R and S in a ratio of 1:1). The activity of Methoprene is restricted to the S enantiomer (S-Methoprene). Since S-Methoprene is the active isomer in the racemic mixture and as they are present in a 1:1 ratio it is possible to extrapolate the results obtained in the studies with Methoprene to S-Methoprene.

Study 1: A three generation reproduction study of Altosid™ in rats. Killeen, J.C. (1974) - CAR IIIA 6.8.2

Methoprene technical was administered to 3 groups of 20 Long-Evans rats sex/group at concentrations of 0, 500 and 2500 ppm (equivalent to 0, 16.3 and 261.6 mg/kg bw/day Methoprene technical and 0, 8.15 and 130.8 mg/kg bw/day S-Methoprene) over a three generation period. This study was not performed according to guidance but has been compared to OECD Guideline 416.

During the growth period, animals from both sexes in the F_0 and F_1 generations showed lower mean body weights and mean weight gains than controls at 2500-ppm dose level. In the F_2 generation, mean body weights were lower than control at the 500 and 2500-ppm dose level groups. However, mean weight gains were comparable. Results are summarised in Table 45.

Table 45: Summary Mean Body Weights – growth periods (g)

Dose level ppm	Growth period												Weight gain
	Initial	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Initial–Week 9/10
F_0 generation - males													
0	79.0	113.3	149.9	188.0	227.8	257.4	286.4	308.6	328.6	345.4	361.9	-	282.9
500	79.1	115.9	152.8	196.1	238.8	264.8	287.1	309.7	329.3	345.9	360.7	-	281.6
2500	79.1	116.5	151.4	192.0	233.6	259.9	282.2	301.3	321.9	338.9	351.4	-	272.3
F_1 generation – males													
0	127.5	-	169.3	211.7	254.3	290.2	315.5	312.0	300.3	346.1	371.9	384.6	257.1
500	121.0	-	159.5	200.2	240.0	276.0	301.9	304.9	294.7	330.5	355.1	371.2	250.2
2500	122.0	-	159.8	203.2	241.3	276.8	302.5	308.2	293.9	325.9	346.3	365.9	243.9
F_2 generation – males													
0	155.5	-	192.6	244.3	281.6	315.2	337.3	367.1	383.5	396.2	404.5	392.0	236.5
500	131.9	-	172.2	211.7	252.2	281.4	298.8	325.7	344.0	357.3	366.1	366.9	235.0
2500	139.2	-	a	222.3	257.7	285.6	299.2	323.5	349.2	364.5	369.6	376.8	237.6
F_0 generation – females													
0	71.1	103.6	131.4	155.9	180.1	193.6	201.6	212.4	223.6	229.2	238.0	-	166.9
500	71.1	100.6	128.8	154.6	178.1	188.9	196.7	207.5	219.5	225.2	233.2	-	162.1
2500	71.1	99.7	126.7	149.9	174.4	185.7	194.6	203.6	215.2	220.6	227.1	-	156.0
F_1 generation – females													
0	113.2	-	134.7	158.7	182.2	200.0	211.9	213.7	209.3	224.9	231.5	243.8	130.6
500	110.5	-	131.0	156.3	175.9	193.9	205.0	209.8	203.1	223.3	226.9	241.1	130.6
2500	108.6	-	131.5	155.4	174.8	192.4	204.8	207.3	214.3	223.9	228.1	235.4	126.8
F_2 generation – females													
0	126.1	-	148.6	170.0	186.0	201.8	213.0	226.3	234.1	239.1	244.6	247.0	120.9
500	121.6	-	146.9	165.6	182.6	196.8	202.8	218.8	228.6	232.7	237.5	237.5	115.9
2500	114.2	-	137.8	156.8	172.4	188.6	195.5	208.6	215.8	222.0	225.5	232.2	118.0

[a]Not recorded

During gestation and lactation, decreased maternal mean body weights were observed at 2500-ppm dose level throughout all generations. In the F_2 generation decreased maternal body weights were also observed at 500-ppm dose level. These effects were not considered to be treatment related. Results are summarised in Table 46.

Table 46: Summary Mean Maternal Body Weights (g)

Dose level (ppm)	Premating	Gestation				Weight gain	Lactation				Weight gain
		Day 0	Day 6	Day 15	Day 20	Gestation 0-20	Day 0	Day 4	Day 14	Day 21	Lactation 0-21
F_0 Mating											
0	238.0	240.3	260.9	301.1	358.3	118.1	286.7	292.8	301.9	293.2	6.5
500	233.2	239.5	261.6	303.8	363.3	123.8	295.9	302.6	311.8	303.9	8.1
2500	227.1	235.3	258.2	297.7	348.0	112.7	286.1	290.4	301.5	296.5	10.4
F_1 Mating											
0	243.8	251.2	279.1	309.8	368.8	117.6	301.3	307.1	308.1	300.3	-0.1
500	241.1	251.2	274.7	311.3	368.8	117.6	297.1	300.3	302.0	301.9	4.8
2500	235.4	244.0	269.4	304.2	357.6	113.6	292.7	307.3	313.3	291.4	-1.3
F_2 Mating											
0	247.0	252.4	282.4	316.4	370.9	118.1	299.0	310.4	310.4	311.6	12.6
500	237.5	245.7	269.0	303.5	356.1	110.4	296.8	298.6	306.3	309.7	12.9
2500	232.2	236.4	262.2	293.8	343.4	107.0	280.1	290.3	291.9	296.0	15.9

In the F_2 mating (F_3 generation), there was a statistically significant decrease in the percentage of live pups born at 2500-ppm dose level. This difference was due to two litters, which contained large numbers of stillborn pups and was not considered to be treatment related. Results are summarised in Table 47. Slightly decreased mean offspring weights were observed at 2500-ppm dose level in the F_1 mating (F_2 generation) on Day 21 of lactation and in the F_2 mating (F_3 generation) throughout the lactation period. Results are summarised in Table 47.

Table 47: Summary of Survival and Growth of Offspring

Dose level ppm	Mean gestation length	Mean No. born/litter		% Gestation survival	Mean No. weaned/litter	% Postnatal offspring survival Days			% Litters with offspring deaths	% Litters weaned	Mean live offspring weights (g)			
	Days	Alive	Dead	Alive/total born		0-4	4-14	4-21	Days 0-21		Day 0	Day 4	Day 14	Day 21
F_0 Mating (F_1)														
0	22.0	11.3	0.1	99.4	9.6	92.8	98.0	98.0	25.0	93.8	5.988	9.070	25.168	36.486
500	21.9	10.9	0.1	99.4	8.4	96.0	97.1	97.1	31.3	100.0	5.838	8.726	26.546	38.319
2500	22.1	10.0	0.3	97.4	8.6	95.3	86.5**	86.5**	31.6	89.5	6.061	8.995	24.345	35.501
F_1 Mating (F_2)														
0	22.3	10.7	0.1	99.0	8.5	89.6	91.9	91.9	72.2	88.9	6.214	9.091	25.788	39.536
500	22.1	11.3	0.5	96.0	8.1	78.1**	89.6	89.6	70.6	88.2	5.785	7.946	23.484	37.101
2500	22.4	10.9	0.3	97.4	8.7	85.4	93.1	90.3	58.8	88.2	6.125	8.666	24.116	35.885
F_2 Mating (F_3)														
0	22.1	10.2	0.4	96.0	8.7	97.2	99.2	99.2	21.4	100.0	6.184	10.421	29.044	39.635
500	21.9	10.4	0.1	99.4	8.8	99.4	100.0	99.3	12.5	100.0	6.051	9.858	27.784	39.477
2500	22.1	9.7	1.2	88.8*	9.0	99.4	97.0	97.0	15.8	94.7	5.886	9.412	25.457	35.967

Significantly different from control:
* ($p<0.05$)
** ($p<0.01$)

At necropsy, no treatment related findings were reported in the F_3 generation. Based on the effects observed on the body weights in adult animals and offspring at 2500-ppm dose level, the NOEL for reproductive toxicity was established to be 500 ppm (equivalent to 16.3 mg/kg bw/day). An observed NOEL for S-Methoprene of 8.15 mg/kg bw/day is established.

Conclusion: Methoprene technical (86.9 and 87.5%) was administered in the diet to two generations of Long-Evans rats at two dose levels of 500 and 2500 ppm (equivalent to 8.15 mg/kg bw/day and 130.8 mg/kg bw/day S-Methoprene).

Overall, minimal and insufficient parental toxicity was demonstrated in this study. The only effects were slight reduction in mean pup weights seen at day 21 of lactation in the F2 generation and throughout lactation in the F3 generation. In conclusion, a clear NOEL of 500 ppm (equivalent to 8.15 mg/kg bw/day S-Methoprene) was established.

The study demonstrated there was no evidence of an adverse effect on reproduction. However it should be noted that insufficient parental effects and toxicity were demonstrated and only two doses were used. Ultimately, it is felt that the dosing parameters for testing the substance were not extended sufficiently to produce the required parental toxicity and as a consequence this substance was not assessed to its full extent.

4.10.2.2 Human information

None available.

4.10.3 Other relevant information

4.10.4 Summary and discussion of reproductive toxicity

Developmental Toxicity: Rat

The teratogenicity of S-Methoprene Technical was investigated by oral administration to 4 groups of pregnant female rats at the following concentrations: 0, 60, 250 and 1000 mg/kg bw/day.

Maternal toxicity was evidenced at the high dose by a statistically significant reduction in food consumption and mean weight gain. There was also a statistically significant reduction in post-implantation loss at this dose level. A NOEL of 250 mg/kg/day was established for both maternal and developmental toxicity.

Developmental Toxicity: Rabbit

In the rabbit developmental study severe maternal toxicity (death, weight loss) was accompanied by significant foetolethality (abortions) and foetotoxicity (runts and retarded ossification) at the high dose of 1000 mg/kg bw/day. An NOEL was established for maternal and foetal toxicity at 100-mg/kg bw/day. Ultimately, the dosing in the rabbit developmental study is considered inadequate. The top dose is considered to be inappropriately high and the mid-range dose provides an NOEL value. However, this NOEL value of 100mg/kg bw/day is used as the NOAEL value and is used to establish a systemic AEL acute reference value even though it may be a more conservative value than what may have been achieved if the study dosing was more appropriately considered.

Fertility: Rat

Methoprene technical was administered in the diet to two generations of Long-Evans rats at two dose levels of 500 and 2500 ppm (equivalent to 8.15 mg/kg bw/day and 130.8 mg/kg bw/day S-Methoprene). Overall, minimal and insufficient parental toxicity was demonstrated in this study. The only effects were slight reduction in mean pup weights seen at day 21 of lactation in the F2 generation and throughout lactation in the F3 generation. In conclusion, 500 ppm (8.15 mg/kg bw/day) was a clear NOEL.

The study demonstrated there was no evidence of an adverse effect on reproduction. However it should be noted that insufficient parental effects and toxicity were demonstrated and only two doses were used. Ultimately, it is felt that the dosing parameters for testing the substance were not extended sufficiently to produce the required parental toxicity and as a consequence this substance was not assessed to its full extent.

4.10.5 Comparison with criteria

The criteria for classification as Cat 1A (H360D May damage the unborn child) are as follows:

'....known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance in Category 1A is largely based on evidence from humans.'

The criteria for classification as Cat 1B (H360D May damage the unborn child) are as follows:

'....clear evidence of an adverse effect on (sexual function and fertility or on) development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects'.

The criteria for classification as Cat 2 (H361D Suspected of damaging the unborn child) are as follows:

'....some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if

occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.'

In accordance with the CLP Regulation (EC) No. 1272/2008 classification criteria for reproductive toxicants, S-Methoprene does not classify for developmental toxicity or teratogenicity. S-Methoprene was determined not to affect fertility in the rat in a 2-generation study.

4.10.6 Conclusions on classification and labelling

In accordance with the classification criteria of the CLP Regulation (EC) No. 1272/2008 and the Guidance to Regulation (EC) No. 1272/2008 on Classification, Labelling and Packaging of substances and mixtures), S-Methoprene does not warrant classification as a reproductive or developmental toxicant.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for reproductive toxicity on the basis of the following findings:

- The developmental toxicity findings in rats, showing at 1000 mg/kg bw/d a statistically significant increase in post-implantation loss (by mistake referred to as a reduction in implantation loss in the CLH report) that appeared concurrently with maternal toxicity (reduction in food consumption and mean body weight)

- The developmental toxicity findings in rabbits, showing at 1000 mg/kg bw/d significant foetolethality and fetotoxicity that appeared concurrently with severe maternal toxicity (mortality and weight loss)

- A 3-generation reproduction study in rats showing slight reductions in mean pup weight in the F_2 and F_3 generation, although concurrently with minimal parental toxicity.

Comments received during public consultation

No comments were received during public consultation on this hazard class.

Assessment and comparison with the classification criteria

The CLH report contained three different studies for assessing the toxicity to reproduction (a fourth available study was considered invalid by the DS because of serious methodological and reporting inadequacies).

Teratology study of test item S-methoprene technical in rabbits (Kolep, 2008; Document IIIA 6.8.1(2) in S-methoprene CAR)

Four groups of 25 inseminated female NZW albino rabbits were dosed, once daily, between days 6 to 27 of presumed gestation by the oral route with S-methoprene at dose levels of 0, 25,100 and 1000 mg/kg bw/d.

The maternal reproductive parameters, overall pregnancy rate, number of corpora lutea and number of implantations were similar in all groups. No treatment related changes were observed at the histopathological examination.

In all the tables in this section below, statistical significance is indicated by either * ($p<0.05$) or ** ($p<0.01$)

The main maternal effects are summarised in the following table:

DOSE	EFFECT
1000 mg/kg bw/d	Body weight: 90% of control by day 28** Body weight gain: 24% of control in the 0-28 day period** Gravid uterine weight: 81% of control* Corrected body weight: 92% of control** 1 mortality after abortion by day 17 6 abortions (5 after organogenesis by day 18) 2 cases of vaginal bleeding 1 case of abnormal bleeding 6 cases of soft faeces
100 mg/kg bw/d	4 cases of soft faeces
25 mg/kg bw/d	1 abortion by day 21 1 case of vaginal bleeding 3 cases of soft faeces
Control	2 mortalities (bacterial infection related) 1 case of vaginal bleeding

The number of viable foetuses in the treated groups was comparable to controls. The results of the external, visceral and skeletal examinations are summarised in the table below:

		DOSE GROUP			
		Control	25 mg/kg bw/d	100 mg/kg bw/d	1000 mg/kg bw/d
Litters examined		18	22	20	17
EXTERNAL EXAMINATION					
Foetuses examined	N	161	181	179	139
Foetuses with abnormalities	%	8	4	9	**21****
Variation	%	7	3	7	**19****
Malformation	%	1	2	2	1
Retarded in bodyweight	%	4	2	6	**11****
Retarded in crown-rump weight	%	4	2	6	**16****
VISCERAL EXAMINATION					
Foetuses examined	N	161	181	179	139
Foetuses with abnormalities	%	19	20	18	28
Variation	%	17	18	17	27

Malformation	%	2	3	1	1
SKELETAL EXAMINATION					
Foetuses examined	N	152	181	171	139
Foetuses with abnormalities	%	13	10	11	**22***
Variation	%	7	8	8	**19****
Malformation	%	5	2	3	3
FOETUSES WITH VARIATIONS					
	%	27	27	27	**49****
FOETUSES WITH MALFORMATIONS					
	%	6	4	5	2

In conclusion, significant foetolethality (abortions) and foetotoxicity (percentages of foetuses with abnormalities, variations, retarded in bodyweight and retarded in crown-rump weight lower than control), concurrent with severe maternal toxicity (reductions in body weight, body weight gain, gravid uterine weight and corrected body weight and diarrhoea), appeared at the high dose of 1000 mg/kg bw/d. Lower doses showed neither maternal nor foetal toxicity.

Teratology study of test item S-methoprene technical in rats (Kolep, 2009; Document IIIA 6.8.1(2) in the CAR for S-methoprene)

The teratogenicity of S-methoprene was investigated by oral administration to 4 groups of pregnant female rats at the following doses: 0, 60, 250 and 1000 mg/kg bw/d.

The number of sperm positive females in the study was 97. None of the females displayed adverse clinical signs and all females survived until necropsy on gestation day 20. No treatment related effects were noted at the necropsies in any group. The main maternal effects are summarised in the following table.

DOSE	**EFFECT**
1000 mg/kg bw/d	Body weight gain: 55% of control over days 17-20 of gestation** Body weight gain: 84% of control over days 0-20 of gestation* Food consumption reduced around 10% during the whole treatment period*
250 mg/kg bw/d	4 non-pregnant animals 18 dams with more than 5 live foetuses
60 mg/kg bw/d	2 non-pregnant animals 19 dams with more than 5 live foetuses

Foetal body weight was not influenced by treatment with the test substance. No treatment related foetal external abnormalities or visceral examinations were observed in the foetal litter. None of the skeletal malformations were significantly increased in the treatment groups.

The main embryo and foetal effects are summarised in the following table:

DOSE	**EFFECT**
1000 mg/kg bw/d	Statistically significant increase in post-implantation loss (but no value was reported in the CLH report) No treatment related effect observed on either the type or incidence of malformations or variations 25% dams with malformed foetuses 70% of females were pregnant

250 mg/kg bw/d	Pre-implantation loss statistically significantly increased (13%, $p<0.05$), but remained below the historical control level (20%) No external or visceral malformations One foetus displayed signs of vertebral abnormalities (having lumbar vertebrae malformations). It was was not considered to be treatment related 11% dams with malformed foetuses 82% of females were pregnant
60 mg/kg bw/d	Incomplete ossification of the skull was noted in 3 foetuses Marked wavy ribs with or without complete ossification were found in 2 foetuses Vertebral abnormalities were found in 3 foetuses No visceral malformations. 37% dams with malformed foetuses 91% of females were pregnant
Control	Marked wavy ribs with or without complete ossification were found in 6 foetuses Vertebral abnormalities were found in 3 foetuses. 39% dams with malformed foetuses 89% of females were pregnant

In conclusion**,** a statistically significant increase in post-implantation loss was evidenced at 1000 mg/kg bw/d concurrently with maternal toxicity (a statistically significant reduction in food consumption and mean weight gain).

Three generation reproduction study of Altosid™ in rats (Killeen, 1974, Document IIIA 6.8.2 in the CAR for S-methoprene)

Methoprene was administered to 3 groups of 20 Long-Evans rats sex/group at concentrations of 0, 500 and 2500 ppm (equivalent to 0, 16.3 and 261.6 mg/kg bw/d methoprene technical and 0, 8.15 and 130.8 mg/kg bw/d S-methoprene) over three generations.

The main adverse effect are summarised in the following table:

DOSE	EFFECT
2500 ppm	Lower mean body weight (not statistically significant) in F_0, F_1 and F_2 males and females Decrease in maternal body weight (not statistically significant) in F_0, F_1 and F_2. Reductions in % of postnatal offspring survival (86.5% versus 98% in control)** in F_1
500 ppm	Lower mean body weight (not statistically significant) in F_2 males and females Decrease in maternal body weight (not statistically significant) in F_2

In conclusion, the only noteworthy alteration was the reduction of 12% in postnatal offspring survival detected in the F_1 generation at the highest dose, where no significant maternal toxicity was reported.

Comparison with the criteria

Overall, the available information yields the following conclusions:

- Foetuses had external and skeletal abnormalities that appeared only in the presence of maternal toxicity in rabbits at doses of 1000 mg/kg bw/d. Doses causing no maternal toxicity did not induce developmental alterations.

- Increases in post-implantation loss (incidence not reported but statistically significant) only in the presence of maternal toxicity in rats at dose of 1000 mg/kg bw/d. Doses causing no maternal toxicity did not induce reproductive alterations.

- Twelve percent reduction in F_1 post-natal offspring survival in rats exposed to 130.8 mg/kg bw/d of S-methoprene.

According to the CLP criteria classification in Category 1A must be based on evidence from human data, which were not present in the CLH report. Therefore, classification as Repr. 1A is not warranted.

Categories 1B and 2 are reserved for presumed and suspected human reproductive toxicants, respectively, and must be based on the presence of clear (Category 1B) or some (Category 2) evidence of alterations in sexual function, fertility, or development. In addition, such evidence must be present in the absence of other toxic effects (or if occurring together with other toxic effects the adverse effects on reproduction must be considered not to be a secondary non-specific consequence of the other concurrent toxic effects).

RAC notes that:

- The only significant alterations in developmental toxicity in rabbits (foetuses with external and skeletal abnormalities) were found in the presence of severe maternal toxicity (reductions of 76, 10, 19 and 8%, in bodyweight gain, body weight, gravid uterine weight and corrected body weight, respectively). These severe reductions could also be responsible of the reported abortions. Thus, these developmental effects in rabbits should not be considered for classification. RAC also highlights that more detailed information about the types of malformations found in exposed rabbit foetuses would have been highly desirable.

- The only significant alterations in reproductive performance in rats (increases in post-implantation loss) were found in the presence of maternal toxicity (statistically significant reductions in bodyweight gain during gestation days 17-20 (by 44%) and 0-20 (by 16%) and in the food intake (by 9%)).

- RAC also highlights the poor reliability of the teratogenicity study in rats, due mainly to the high background incidences and absence of a dose-response relationship in the number of foetuses with malformations (39% in control versus 37, 11 and 25% for 60, 250 and 1000 mg/kg bw/d, respectively) and to the absence of information about the types of malformations found.

- The reduction in the F_1 post-natal offspring survival in rats exposed to 130.8 mg S-methoprene/kg bw/d was not found in the other two generations, which suggests, together with the low incidence of the effect, that it is incidental and not treatment related and therefore does not justify classification.

In conclusion, RAC agrees with the DS proposal for no classification of S-methoprene for reproductive toxicity.

Supplemental information – In-depth analyses by RAC

During the RAC Plenary session, the DS supplied new information to cover gaps in reporting of the teratology study in rats. Specifically, the following information was considered by RAC:

- A statistically significant ($p<0.05$) reduction in early embryonic death at the dose of 250 mg/kg bw/d (2% incidence versus 6% incidence in controls). No other dose level caused significant alterations in this parameter. The alterations in early embryonic death are of no toxicological significance and therefore cannot be considered relevant for classification.
- An increase of 4-fold in the late embryonic deaths in animals exposed to 1000 mg/kg bw/d. However, RAC notes that the incidence of embryonic deaths at this dose (4%) was still within the historical control range and thus was not sufficient for fulfilling the criteria of classification.
- The above stated increase in the post-implantation loss was of 14%, while the incidence of this finding in control animals was 7%.

RAC does not consider that the incidence of any of these developmental findings in rats are sufficient for classification of S-methoprene as toxic to reproduction category 2.

4.11Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity

There was no indication of neurotoxicity such as behavioural changes or neurological disturbances from the standard systemic toxicity studies conducted. This includes any single or repeat dose studies conducted. In addition, neurotoxicity studies are not a standard requirement as they are only required for substances of similar or related structures to those capable of inducing delayed neurotoxicity such as organophosphates.

4.11.1.2 Immunotoxicity

No data.

4.11.1.3 Specific investigations: other studies

No data.

4.11.1.4 Human information

No data.

4.11.2 Summary and discussion

No data.

4.11.3 Comparison with criteria

No data.

4.11.4 Conclusions on classification and labelling

No data.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

5.1.1 Stability

Table 48: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis			
OECD 111	S-Methoprene technical was found to be **hydrolytically stable** at pH 4, 7 and 9 at all temperatures examined. (25, 37 and 50). At pH 1.2 hydrolysis of the test material was rapid with a DT_{50} value of 17 hours.	The study was considered acceptable with a Reliability score of 2.	Laky, V. (2002a), Hydrolysis of S-Methoprene technical as a function of pH. Toxicological Research Centre Ltd., H-8200 Veszprém-Szbadságpuszta, P.O.Box 348, 8201, Hungary, unpublished report no.: 01/616-336AN. CAR IIIA 7.1.1.1.1
Photolysis in water			
OECD Draft Guideline: Phototransformation of Chemicals in Water-Direct and Indirect Photolysis (August 2000)	The aqueous photolysis study performed under lab conditions is not representative of natural conditions and highly overestimates the degradation potential of S-Methoprene and the levels of metabolites (15 d continuous irradiation with a Xe lamp, pH 7, sterilised, 22 ± 2 ºC). Under field conditions, **photolysis in water may only be relevant in the upper few centimetres of a water body**.	The study was considered to be acceptable but yielded uncertain results. Reliability score of 2.	McCorquodale, G. (2009), Photodegradation of [^{14}C]-S Methoprene, Charles River, Tranent, Edinburgh, EH33 2NE, UK. Unpublished report no: 807299. CAR IIIA 7.1.1.1.2-1

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Table 49: Summary of relevant information on biodegradation

Method	Results	Remarks	Reference
Biodegradation			
OECD 301D	S-Methoprene technical, at a concentration of 2 mg/l and 8 mg/l, attained 49.45% and 20.99% degradation, respectively, after 28 days. The concentration of 8 mg/L is exceeds the water solubility of S-Methoprene (6.85 mg/L). The results observed at 2 mg/L are considered reliable. S-Methoprene is not readily biodegradable.	The study was considered acceptable with a Reliability score of 2.	Gáty, S. (2002a), Determination of biodegradability of S-Methoprene Technical test item with closed bottle test. Toxicological Research centre Ltd., H-8201 Veszprém, Szabadságpuszta, P.O. Box 348, Hungary, unpublished report no.:01/616-322AN. CAR IIIA 7.1.1.2.1
OECD 302C	The percentage biodegradation of S-Methoprene reached a mean of 4.2 % after 7 days, 24.5 % after 14 days, 77.5 % after 21 days and 85.8 % after 28 days.	The study was considered acceptable with a Reliability score of 1.	Dr. Vértesi, A. (2014); Inherent Biodegradability of S-Methoprene In Modified MITI Test (II). TOXI-COOP ZRT, 8230 Balatonfüred, Arácsi út 97, Hungary , unpublished report No.: 484.462.3617

This closed bottle test concluded that S-Methoprene is not readily biodegradable. The OECD guideline states "*If in a toxicity test, containing both the test substance and a reference compound, less than 35% degradation (based on total DOC) or less than 25% (based on total ThOD or ThCO2) occurred within 14 days, the test substance can be assumed to be inhibitory (see Annex II for other toxicity tests). The test series should be repeated, using a lower concentration of test substance (if this can be done without seriously impairing the accuracy of the DOC determination) and/or a higher concentration of inoculum, but not greater than 30 mg solids/l.".* At the higher test concentration of 8 mg/L less degradation took place relative to tests performed at 2 mg/L. This suggests a concentration effect. The study does not report the concentration of test item used in the toxicity test. If the test was performed at a low concentration of test item, negligible inhibition may take place resulting in the observed result in the tox control. The CA notes the OECD 301 guideline states *"If*

inhibition due to toxicity is to be avoided, it is suggested that the test substance concentrations used in ready biodegradability testing should be less than 1/10 of the EC_{50} values (or less than EC_{20} values) obtained in toxicity testing)". For S-Methoprene the EC_{50} for activated sludge is reported as >100 mg/L (3 hr). The test concentrations used in the experiment were 2 mg/L and 8 mg/L. The higher test concentration of 8 mg/L lies above the water solubility. The results at 2 mg/L maybe more reliable than the results at 8 mg/L. The validity criteria for the test were fulfilled.

The modified MITI (II) test showed >70% degradation within 28 days. This represents inherent biodegradability (as specified in TGD). The failure to reach 70% within 14 days means that the specific inherent biodegradability criteria were not met and therefore that extrapolation of the results for use in STP models is not possible.

5.1.2.2 Screening tests

Not relevant to this dossier

5.1.2.3 Simulation tests

Not relevant to this dossier

5.1.3 Summary and discussion of degradation

S-Methoprene is not considered to be readily biodegradable and it is hydrolytically stable at environmentally relevant pH values. Photolysis may only be relevant in the upper few centimeters of a water body. These findings are appropriately reflected in the classification and labelling of S-Methoprene.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 50: Summary of adsorption/desorption

Method	Results	Remarks	Reference
Adsorption and mobility in soil			
OECD 106	S-Methoprene is readily adsorbed to and desorbed from the soil. With K_{oc} values of 537, 684 and 1407 in three soil types and an average of 876, S-Methoprene is classified as being of low mobility according to the McCall and UK Soil Survey and Land Research Centre Pesticide Mobility classification systems. .	The study was considered acceptable with a Reliability score of 1.	Laky, V. (2002b), Adsorption/desorption test of S-Methoprene technical. Toxicological Research Centre Ltd., H8200 Veszprém-Szabadságpuszta, P.O.Box 378, 8201, Hungary, unpublished report no.: 01/616-331TL. CAR IIIA 7.2.3.1

No further studies were submitted due to the use pattern proposed for S-Methoprene and the indication that based on the above study that S-Methoprene exhibits low mobility in soil. The three soil types used in the study with the corresponding distribution coefficients are described below.

Table 51: Summary of soil types used in study

Soil type	Type 1	Type 2	Type 3
Sand (%)	14.7	48.9	88.96
Clay (%)	22.2	18.1	5
Organic carbon (%)	1.16	1.21	-
pH	7.31	5.65	4.64
K_d (L/kg)	7.9	6.5	5.5
K_{oc} (L/kg)	684	537	1407
1/n	-	-	-
r^2	-	-	-

5.2.2 Volatilisation

Not relevant for this dossier. The vapour pressure of (3.15 mPa) and molecular weight (310.5) of S-Methoprene allow that it will not readily volatilise into the atmosphere at ambient temperature and pressure.

5.2.3 Distribution modelling

Table 52: Summary of distribution modeling

Method	Results	Remarks	Reference
Mackay Level I fugacity model applying Type 1 type chemical partitioning	S-Methoprene was predicted to partition predominantly to soil (97.8%) and to a much lesser extent to sediment (2.2%). Insignificant amounts are anticipated to be distributed to suspended sediment (0.07%), to air (0.000001%), to fish (0.006%), to water (0.00003%) and to aerosols in the atmosphere (0.00000004%). S-Methoprene has a fugacity value of 3.03×10^{-11} Pa and is found to partition predominantly to soil.	The modeling was considered acceptable with a Reliability score of 1.	Laky, V. (2002b), Adsorption/desorption test of S-Methoprene technical. Toxicological Research Centre Ltd., H8200 Veszprém-Szabadságpuszta, P.O.Box 378, 8201, Hungary, unpublished report no.: 01/616-331TL. CAR IIIA 7.2.3.1

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

For S-Methoprene (CAS: 65733-16-6) calculations using BCFBAF, the CAS number is provided to the software and the SMILES structure are generated automatically. Also the partition coefficient value is also provided, i.e. Log Kow 6.34. For this model other physical-chemical properties are not necessary as they are not part of this specific model calculation. The calculated BCF is 516. This result is consistent with literature values of the BCF of S-Methoprene. The UK Pesticide Database and the US EPA Integrated Pest Management Plan, 2006, both report a BCF of 457 for S-Methoprene. According to CLP Regulation (EC) No. 1272/2008 the bioconcentration factor (BCF) threshold limit for classification purposes is 500. From the calculation the BCF of S-Methoprene is 516, therefore, based on these results, S-Methoprene meets B criterion (CAR IIIA7.4.2.1). Please note that according to the criteria for the PBT assessment for Annex XIII to Regulation (EC) 1907/2006 S-Methoprene does not meet the B criterion (>2000).

5.3.1.2 Measured bioaccumulation data

No studies were performed on bioaccumulation.

5.3.2 Summary and discussion of aquatic bioaccumulation

The calculated bioaccumulation factor (BCF) of 516 marginally exceeds the threshold limit for classification purposes under Regulation (EC) No. 1272/2008. Therefore S-Methoprene is considered to have potential to bioaccumulate.

5.4 Aquatic toxicity

Table 53: Summary of relevant information of S-Methoprene technical on aquatic toxicity (text highlighted in bold indicates key study)

Method	Results	Remarks	Reference
OECD 203	96 hr LC_{50}=4.26 mg/L (measured)	Lowest endpoint in this study	CAR, IIIA7.4.1.1/01. Gáty, S (2002a). Fish acute toxicity study S-Methoprene technical test item on Zebrafish, Toxicological Research Centre Ltd., Report No. 01/616-009H, GLP (unpublished).
OECD 202 ; Comm Reg. (EC) No 440/2008 ; EPA Guideline 712-C-96-114	**48 hr EC_{50}=0.22 mg/L (measured)**	**24 hr EC_{100}=0.66 mg/L (measured)**	**CAR, IIIA7.4.1.2/02. Istvan, A.(2012) Acute Toxicity of S-Methoprene on *Daphnia magna* in a 48-hour Acute Immobilisation Test, TOXI-COOP ZRT., 8230 Balatonfüred, Arácsi út 97 , Hungary, report no.: 484.441.3614 (unpublished).**
OECD 202	48 hr LC_{50}=0.38 mg/L (nominal)	Supporting study	CAR, IIIA7.4.1.2/01. Gáty, S. (2002d) Acute immobilisation test with S-Methoprene technical in Daphnia magna, Toxicological Research Centre Ltd., Report No. 01/616-023DA, GLP (unpublished).
OECD 211	**21 d NOEC=0.019 mg/L (measured)**	**Most sensitive long-term endpoint**	**CAR, IIIA7.4.3.4/01. Istavan, A. (2012) Chronic Toxicity of S-Methoprene**

			to *Daphnia magna* in a 21-day Reproduction Test, TOXI-COOP ZRT., 8230 Balatonfüred, Arácsi út 97 , Hungary report no.: 484.447.3615 (unpublished).
OECD 201	72 hr E_rC_{50}=2.264 mg/L (nominal)	No remarks	CAR III, 7.4.1.3/01. Hernádi, D. (2002) Algal growth inhibition test with S-Methoprene technical. Toxicological Research Centre Ltd., Report No. 01/616-022AL, GLP (unpublished).
OECD 209	3 hr EC_{50}>100mg/L	Static respiration inhibition test	CAR, IIIA7.4.1.4/01 Gáty, S. (2002c) Activated sludge, respiration inhibition test with S-Methoprene technical test item. Toxicological Research Centre Ltd., Report No. 01/616-027AS, GLP (unpublished).

Note: Methoprene is a terpenoid consisting of a racemic mixture of two enantiomers (R and S in a ratio of 1:1). The activity of Methoprene is restricted to the S enantiomer (S-Methoprene). Since S-Methoprene is the active isomer in the racemic mixture and as they are present in a 1:1 ratio it is possible to extrapolate the results obtained in the studies with Methoprene to S-Methoprene.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Nominal test concentrations in the static study (A7.4.1.1) were 0.63, 1.25, 2.50, 5.00 and 10.00 mg/L. The concentration of the test material was not measured, before, after or during the course of the experiment. Observations of fish were carried out at 3, 6, 24, 48, 72, and 96 hr. Mortality and sub-lethal effects (fast motility of operculum, decreased activity, localisation near bottom of the aquarium, darkening of body colour and eyes) were recorded at sampling times. No adverse effects were noted

in the controls or the two lowest test concentrations. The LC_{50} values were calculated by probit analysis with 95 % confidence limits using TOXSTAT 3.5 statistical software. At 2.50 mg/L fast motility of operculum, decreased activity, localisation near bottom of the aquarium were recorded. At the highest two concentrations these effects in addition to the darkening of body colour were recorded. The highest concentration used producing no mortality was 1.25 mg/L and the lowest concentration producing 100 % mortality was 10.00 mg/L. The study has a reliability of 2 due to the minor deviation from the OECD Guideline 203, whereby test concentrations were not measured. S-Methoprene is acutely toxic to fish, with the LC_{50} at 96h of 4.26 mg/L.

5.4.1.2 Long-term toxicity to fish

No long-term toxicity study was submitted. Not relevant for this dossier.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The key study on Daphnia magna (A7.4.1.2.2) was conducted in semi-static conditions because it is an adsorbing substance, of the test item. All vessels were preconditioned for at least two days prior to test initiation using solutions of the test substance in order to minimise the concentration decline during the experiment. Water renewal periods were 24 hours. The measured test concentrations (geometric mean of four replicates) were 0.07, 0.12, 0.22, 0.40 and 0.66 mg/L. Immobilisation of the test animals was recorded at 24 and 48 hr after treatment. At 24 and 48 hr EC_{50} values of the test item and their confidence limits were calculated using Probit analysis by SPSS PC+ software. The 24 hr EC_{50} was > 0.66 mg/L and the 48 hr EC_{50} was calculated to be 0.22 mg/L. The corresponding 48 hr NOEC and EC_{100} were 0.12 and 0.66 mg/L respectively. This study has a reliability of 1. S-Methoprene is acutely toxic to the aquatic invertebrate, Daphnia magna, with the EC_{50} at 48h of 0.22 mg/L.

The supporting study on Daphnia magna (A7.4.1.2.1) had five nominal concentrations of S-Methoprene: 0.15, 0.24, 0.39, 0.63 and 1.00 mg/L. There were two deviations from the OECD Guideline 202: the concentration of the test substance was not measured at the highest and lowest concentrations at the beginning and end of the test and the hardness of the dilution water was not documented. These deviations were not considered to have affected the scientific validity of the study or the interpretation of the results, however, the study has a reliability of 2. The 24 hr EC_{50} of S-Methoprene for Daphnia magna was calculated to be 0.48 mg/L and 48 hrEC_{50} value was calculated to be 0.38 mg/L.

5.4.2.2 Long-term toxicity to aquatic invertebrates

In this semi-static study (A7.4.3.4), the nominal test item concentrations were 0.01, 0.02, 0.04, 0.07, 0.12 and 0.20 mg/L. Test solutions were renewed three times per week. The test solutions were prepared using water miscible solvent (acetone). An untreated control and an additional solvent control group were investigated concurrently. There were 10 replicates per treatment. The concentration of the solvent (acetone) was 0.1 mL/L in each test concentration and in the additional solvent control. The measured test item concentrations deviated more than 20 % from the nominal during the experiment therefore the time-weighted mean of the measured start and end concentrations at each water renewal period were calculated in order to determine exposure concentrations. The calculated time-weighted mean concentrations were the followings: 0.009, 0.019, 0.030, 0.049, 0.074 and 0.131 mg/L. All biological results are based on these measured test item concentrations. The

offspring produced by each parent animal were removed and counted daily from the appearance of the first brood. Mortality was measured daily. Additionally, the length of the parent animals was measured at the end of the test. The validity criteria of the study were fulfilled. The long-term NOEC at 21 d, based on offspring, was statistically determined to be 0.019 mg/L (Bonferroni t-Test, α=0.05). This was also the NOEC for growth measurement. Mortality results did not follow a dose response pattern so these results were excluded from the reproductive output analysis. S-Methoprene is chronically toxic to the aquatic invertebrate, Daphnia magna.

5.4.3 Algae and aquatic plants

5.4.3.1 Short-term toxicity to algae and aquatic plants

In the study on algal growth (A7.4.1.3) nominal test concentrations were 0.0625, 0.1250, 0.5000, 1.000 and 2.000mg/L. There were three vessels per test concentration, six per control group and three per reference concentration. The S-Methoprene concentrations were not analysed throughout the study, however this deviation was not considered to affect the scientific validity of the study. Cell concentrations were determined at 0, 24, 48, and 72 hours. The 0 – 72 hour average specific growth of S-Methoprene at the following concentrations: 0.125, 0.25, 0.5, 1 and 2 mg/L were significantly different from that of the control group. The ErC_{50} was the only endpoint that was documented: ErC_{50} = 2.264 mg/L.

5.4.3.2 Long-term toxicity to algae and aquatic plants

Not relevant for this dossier.

5.4.4 Other aquatic organisms (including sediment)

5.4.4.1 Short-term toxicity to other aquatic organisms (including sediment)

In the study on the inhibition to aquatic microbial activity (A7.4.1.4) the initial test concentrations were nominally 6.3, 12.5, 25, 50 and 100 mg/L. Actual concentrations were not documented. The test parameter was respiration inhibition, based on oxygen consumption rate, in activated sludge. Measurements of oxygen uptake of the activated sludge were performed 3 hours after initiating aeration. The validity criteria for the control respiration rates and reference material EC_{50} values were satisfied. The effect of the test material on the respiration of activated sewage sludge micro-organisms gave a 3 hour EC_{50} of greater than 100 mg/L. Therefore, it can be assumed that S-Methoprene is non-toxic to sewage treatment microbes.

5.4.4.2 Long-term toxicity to other aquatic organisms (including sediment)

Not relevant for this dossier.

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

CLP

S-Methoprene classifies as Acute Category 1 based on the lowest endpoint from acute studies on all three trophic levels: *Daphnia magna*: 48 hr EC_{50}=0.22 ($0.1 < LC_{50} \leq 1$ mg a.s./L). Chronic toxicity was only assessed for aquatic invertebrates, however since this can be expected to be the most

sensitive level the resulting hazard category accurately reflects the chronic risk to aquatic organisms. Therefore, S-Methoprene also classifies as Chronic Category 1 classification: *Daphnia magna*: NOEC = 0.019 mg/L (measured) (0.01 < NOEC ≤ 0.1 mg/L), and is not readily degradable.

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

Based on the requirements of the CLP Regulation (EC) No. 1272/2008; S-Methoprene should be Classified:

Aquatic Chronic 1
H400 Very toxic to aquatic life
H410 Very toxic to aquatic life with long lasting effects
Signal Word: Warning
Pictogram:

The environmental hazard pictogram is required
P273 Avoid release to the environment
P391 Collect spillage
P501 Dispose of contents/container in accordance with applicable regulations
Acute M-factor of 1 is applicable based on 0.1 < LC_{50} ≤1 mg/L.
Chronic M-factor of 1 is applicable based on 0.01 < NOEC ≤ 0.1 mg/L.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

The DS proposed to not consider S-methoprene as rapidly degradable. The basis for this proposal is that S-methoprene was found to be not readily biodegradable in an OECD TG 301D test system (Gáty, 2002a), where a maximum degradation of 49.45% after 28 days was measured. A modified MITI Test (II) (OECD TG 302C) (Vértesi, 2014) failed to reach 70% degradation within 14 days which means that the specific inherent biodegradability criteria were not met. S-methoprene is hydrolytically stable at environmentally relevant pH values (OECD TG 111) (Laky, 2002a).

Aquatic Bioaccumulation

The DS proposed to consider S-methoprene as having the potential to bioaccumulate. The proposal is based on a calculated bioaccumulation factor (BCF) of 516, derived using the BCFBAF software. This value is consistent with literature values from the UK Pesticide Database and the US EPA Integrated Pest Management Plan (2006), which both report a BCF of 457.

Acute Toxicity

The DS proposed to classify S-methoprene as Aquatic Acute Category 1; H400 with an M-factor of 1. The basis for this proposal is that all three trophic levels were tested in acute studies which resulted in:

- 48 h EC_{50} of 0.22 mg/L in an OECD TG 202 test system with *Daphnia magna* (Istvan, 2012)
- 96 h LC_{50} of 4.26 mg/L (measured) in an OECD TG 203 test system with Zebra fish (Gáty, 2002a)
- 72 h E_rC_{50} of 2.264 mg/L (nominal) in an OECD TG 201 algal growth inhibition test (Hernádi, 2002)

Consequently, aquatic invertebrates were found to be the most sensitive species. An M-factor of 1 is applicable based on $0.1 < LC_{50} \leq 1$ mg/L.

Chronic Toxicity

The DS proposed to classify S-methoprene as Aquatic Chronic Category 1; H410 with an M-factor of 1. The basis for this proposal is the following:

- NOEC of 0.019 mg/L (measured) in an OECD TG 211 test system with *Daphnia magna* (Istvan, 2012)

and that S-methoprene is not readily degradable. Chronic toxicity was only assessed for aquatic invertebrates and the DS argued that this can be expected to be the most sensitive level. An M-factor of 1 is applicable based on $0.01 < NOEC \leq 0.1$ mg/L.

Comments received during public consultation

Comments on the proposed classification related to environmental hazards were received from three MSCAs all supporting the classification of S-methoprene as Aquatic Acute 1; H400 M=1, and Aquatic Chronic 1; H410 M=1, as specified in the proposal.

Further, three comments were received from an industry representative submitting information and study reports on degradation of S-methoprene (aerobic degradation in soil and sediments, and inherently biodegradable studies), on metabolism of S-methoprene and on its ecotoxicity towards soil organisms (*Eisenia fetida* and *Collembolan*). The DS replied that all studies were evaluated during the approval process of the biocidal active substance. For the CLP report they were not considered because their results would not have contributed to the classification, according to the criteria in CLP Regulation Annex I 4.1.2.9.5, further developed in the CLP guidance section 4.1.3.2.3.2. The DS did not include the soil ecotoxicity study and RAC did not evaluate it, because soil ecotoxicity studies are in general not relevant for CLP since there are no classification criteria to evaluate them against. Environmental hazard classification is based on aquatic data, namely fish, crustacea and algae or other aquatic plants (see CLP Regulation Annex I 4.1.2.6 and 4.1.2.7).

Additional key elements

During the process of opinion development RAC received comments on S-methoprene from the registrant. In their document "Babolna Bio's Comments against the proposed

classification of S-methoprene as Aquatic Chronic 1", they provided arguments in support of Aquatic Chronic 2. Their main argument was that, based on the simulation biodegradation studies, on the photolysis and on the additional hydrolysis tests, S-methoprene is rapidly degradable in the aquatic environment. However, in their statement the registrant confirmed that S-methoprene is highly insoluble and lipophilic which for RAC has the consequence that degradation tests have to be assessed with special care and expert scientific knowledge.

Assessment and comparison with the classification criteria

Degradation

The Guidance on the Application of the CLP Criteria (section 4.1.3.2.3.2 - Degradation) clearly states that biodegradation screening tests are the preferred data to assess if a substance is rapidly degradable. In the case of a fail in the screening test, other evidence of rapid degradation in the environment may be considered. Further, it is clearly stated that: "*The following decision scheme may be used as a general guidance to facilitate decisions in relation to rapid degradability in the aquatic environment and classification of chemicals hazardous to the aquatic environment.*

A substance is considered to be <u>not</u> rapidly degradable unless at least one of the following is fulfilled:

a. The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability. The pass level of the test (70 % DOC removal or 60 % theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation, if it is possible to evaluate this according to the available test data (the ten-day window condition may be waived for complex multi-component substances and the pass level applied at 28 days, as discussed in point II.2.3 of Annex II to this document). If this is not possible, then the pass level should be evaluated within a 14 days' time window if possible, or after the end of the test; or

b. The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days (corresponding to a degradation of >70 % within 28 days); or

c. *The substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydrolysis, in the aquatic environment with a half-life <16 days (corresponding to a degradation of >70 % within 28 days), and it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment*"

In the CLH Report, the following test results in relation to the criteria (above) can be found:

a. Ready Biodegradability (OECD TG 301D): pass level not achieved (49.45% and 20.99% degradation at 2 and 8 mg/L, respectively)

b. No data

c. S-methoprene is hydrolytically stable under environmentally realistic conditions. However, S-methoprene has a half-life for photolysis < 16 days but it has not been shown that the degradation products do not fulfil the criteria for classification, hence this criterion is not fulfilled.

It can be concluded that 2 out of 3 among the preferred data are available and that they demonstrate that S-methoprene is not rapidly degradable for the purpose of CLP.

In the CLH report (see Table 49) the study "Inherent Biodegradability of S-Methoprene In Modified MITI Test (II)" (Vértesi, 2014) was evaluated by the DS. The study demonstrates a degradation of only 24.5% after 14 days. The DS stated in the CLP report on page 64 that "*The failure to reach 70% within 14 days means that the specific inherent biodegradability criteria were not met and therefore that extrapolation of the results for use in STP models is not possible.*" The DS referred to the specific criteria for "not P" of the Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment. The failure of S-methoprene to meet the "not P" criteria confirms the initial assessment of the substance as "non readily biodegradable". In an inherent biodegradability test, according to OECD TG 302C, the degradation reached 85.8% after 28 days; this may be considered as evidence of inherent biodegradability of S-methoprene, however this test result must not be compared with the specific criteria of the CLP guidance since degradation has to be demonstrated under environmentally realistic conditions to have met the criterion of "rapid degradability". An MITI Test (II) e.g. at 28 °C, especially if modified, *per se* never represents environmentally realistic conditions (e.g. 12 °C).

During public consultation two additional studies on degradation were submitted. The DS replied that these studies had already been evaluated as part of the approval of the biocidal active substance process, and consequently, these studies were available to the DS when writing the CLH report. However, the dossier submitter stated that these studies do not contribute to the classification of S-methoprene and for this reason were not included in the CLH report.

The study "S-Methoprene: Route and Rate of Degradation of [14C]S-methoprene in Aerobic Aquatic Sediment Systems" (CAR for S-Methprene, IIIA 7.1.2.2.2) investigated S-methoprene in two aquatic systems (river and pond) at 20 ± 2 °C in the dark. Since S-methoprene has a strong tendency to adsorb to sediment, such a study design needs expert judgement regarding the validity of the data before using the results for classification purposes. Significant formation of bound residues was observed (36.9 - 41.0%). As a consequence RAC notes that the DT_{50} values from the study report may not represent degradation but only represent dissipation from the observed compartment (e.g. water phase) or the formation of bound residues within the observed compartment (e.g. sediment). Therefore, such a $DisT_{50}$ values must not be compared with the specific criteria in the CLP guidance.

However, the rate of mineralisation may be used as an indication of degradation. The formation of radioactive carbon dioxide was significant, and constantly increased throughout incubation in both systems, reaching maximum mean amounts of 54.9% (river) and 67.5% (pond) of the applied radioactivity after 100 days of incubation at 20 °C. These degradation rates clearly do not meet the CLP criteria of 70% after 28 days at environmentally realistic conditions (e.g. 12 °C) and consequently cannot be evaluated as convincing scientific evidence for rapid degradability, as suggested in the comments by the industry representative.

The study "S-Methoprene: Degradation and Metabolism in Four Soils of [14C] S-methoprene Incubated under Aerobic Conditions" (CAR for S_Methoprene, IIIA 7.2.2.1-2) is in general not relevant for CLP purpose since biotically or abiotically degradation in the aquatic environment needs to be demonstrated.

RAC agrees with the proposal and argumentation (including the response to the comments by the industry representative) of the DS to not consider S-methoprene as rapidly degradable. The basis for this is that S-methoprene is not readily biodegradable, hydrolytically stable at environmentally relevant pH values and environmentally realistic conditions (12 °C).

Aquatic Bioaccumulation

RAC agrees with the proposal and argumentation of the DS that S-methoprene has the potential for bioaccumulation, based on the calculated log K_{OW} greater than 6 and the calculated BCF of 516.

Acute Toxicity

RAC notes that the cited 72-h algal E_rC_{50} of 2.264 mg/L (nominal) is presumably an extrapolation since it is higher than the highest nominal test concentration of 2 mg/L. RAC also notes that test substance concentration was not well maintained in the 21-d *Daphnia* test even though it was semi-static (measured concentrations were ~61-66% of nominal at the top two doses). The CLH report also mentions that special measures were taken to try to minimise losses in the semi-static acute Daphnia study, but no further information is provided for the acute fish or algal studies (both static). RAC therefore assumes it is likely that the actual exposure concentrations in these latter two studies were lower than the reported nominals (there was no analytical verification of concentrations). However, losses would have to have been more than 90% before fish/algae could become as sensitive as Daphnia, so the lack of measured concentration data is in this specific case unlikely to be important.

RAC agrees with the proposal and argumentation of the DS to classify S-methoprene as **Aquatic Acute Category 1; H400, with an M-factor of 1**. The basis for this is the 48 h EC_{50} of 0.22 mg/L in an OECD TG 202 test system with *Daphnia magna* (Istvan, 2012).

Chronic Toxicity

No fish NOEC and no algal NOEC were provided. The CLH report states that in the acute algae study, the 0 – 72 h average specific growth of S-methoprene beginning with 0.125 mg/L (nominal) and for all higher concentrations were significantly different from that of the control group (see 5.4.3.1 on page 70 of CLH report). As a consequence, the NOEC for algae would be assumed to be 0.0625 mg/L (nominal). Again RAC assumes it is likely that the actual exposure concentrations were lower than the reported nominals (there was no analytical verification of concentrations). However, losses would have to have been more than 70% before algae could become as sensitive as Daphnia, so the lack of measured concentration data is in this specific case unlikely to be important.

RAC agrees with the DS that the lowest aquatic chronic toxicity of S-methoprene is the NOEC of 0.019 mg/L (measured) in an OECD TG 211 test system with *Daphnia magna* (Istvan, 2012).

RAC also agrees with the DS that S-methoprene is not rapidly degradable. This would result in a classification of S-methoprene as Chronic Category 1; H410, with an M-factor of 1.

RAC also applied the surrogate approach since studies on chronic fish and chronic algae toxicity were not available. The surrogate approach results (the substance being not rapidly degradable and the algae E_rC_{50} = 2.264 mg/L (Hernádi, 2002) and the fish LC_{50} at 96 h of 4.26 mg/L (Gáty, 2002a)) in a classification of S-methoprene as Aquatic Chronic 2; H411.

Since the most stringent outcome is chosen, RAC concludes to classify S-methoprene as **Aquatic Chronic Category 1; H410, with an M-factor of 1**.

6 OTHER INFORMATION

Not applicable.

7 REFERENCES

8 ANNEXES