Committee for Risk Assessment

RAC

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

Adopted

3 June 2016

3 June 2016

CLH-O-0000001412-86-114/F

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

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

Chemical name: **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**

The proposal was submitted by **Ireland** and received by RAC on **15 July 2015.**

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

PROCESS FOR ADOPTION OF THE OPINION

Ireland has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **28 July 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 September 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Michael Neumann**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **3 June 2016** by **consensus.**

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	607-RST-00-X	isopropyl (2E,4E,7S)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate; S-methoprene	Not available	65733-16-6	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=1	
RAC opinion	607-RST-00-X	isopropyl (2E,4E,7S)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate; S-methoprene	Not available	65733-16-6	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=1	
Resulting Annex VI entry if agreed by COM	607-RST-00-X	isopropyl (2E,4E,7S)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate; S-methoprene	Not available	65733-16-6	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

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.

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.**

HUMAN HEALTH HAZARD EVALUATION

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 4 hours of exposure to a liquid aerosol Single dose of 2.38 mg/L	No mortalities Clinical observations: activity decrease and piloerection in both sexes, red staining around the nose in males. Animals were asymptomatic by Day 1. 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**	Leeper, 1999 Document IIIA 6.1.3 in S-methoprene CAR

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.**

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.**

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.**

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.**

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.**

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 Daily (capsule)	<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. Significant increase in liver weight in males and females.	Török, 2007 Document IIIA 6.1.4/2 in S-methoprene CAR **Conclusion:** Gastrointestinal and hepatic adverse effects at doses of 300 mg/kg bw/d and greater. **Guidance value for classification as Category 2: 100 mg/kg bw/d.**

	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.	
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.**

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**.

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.

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.**

ENVIRONMENTAL HAZARD EVALUATION

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).

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 EC50 of 0.22 mg/L in an OECD TG 202 test system with Daphnia magna (Istvan, 2012)
- 96 h LC50 of 4.26 mg/L (measured) in an OECD TG 203 test system with Zebra fish (Gáty, 2002a)
- 72 h ErC50 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).

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 of the DS (including their response to the comments by the industry representative) not to 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**.

ANNEXES:

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

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