Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Methyl salicylate

EC Number: 204-317-7
CAS Number: 119-36-8

CLH-O-0000006716-67-01/F

Adopted

20 September 2019

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CLH-O-0000006716-67-01/F

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

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

Chemical name: **Methyl salicylate**

EC Number: **204-317-7**

CAS Number: **119-36-8**

The proposal was submitted by **France** and received by RAC on **24 October 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

France 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 **12 November 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **25 January 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ralf Stahlmann**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

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 **20 September 2019** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Methyl salicylate	204-317-7	119-36-8	Repr. 1B Acute Tox. 4 Skin Sens. 1B Aquatic Chronic 3	H360D H302 H317 H412	GHS07 GHS08 Dgr	H360D H302 H317 H412		oral: ATE = 580 mg/kg bw	
RAC opinion	TBD	Methyl salicylate	204-317-7	119-36-8	Repr. 2 Acute Tox. 4 Skin Sens. 1B Aquatic Chronic 3	H361d H302 H317 H412	GHS07 GHS08 Dgr	H361d H302 H317 H412		oral: ATE = 890 mg/kg bw	
Resulting Annex VI entry if agreed by COM	TBD	Methyl salicylate	204-317-7	119-36-8	Repr. 2 Acute Tox. 4 Skin Sens. 1B Aquatic Chronic 3	H361d H302 H317 H412	GHS07 GHS08 Dgr	H361d H302 H317 H412		oral: ATE = 890 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Chemical structure and pharmacological action

Methyl salicylate (MeS) and acetylsalicylic acid (ASA, aspirin) are related substances, both are esters of SA (ortho-hydroxy benzoic acid) which is characterised by a carboxyl group and a hydroxyl group. Salicylic acid (SA) is the common hydrolysis product of both substances.

Both esters are hydrolysed in the mammalian organism. Besides salicylic acid, acetic acid is a hydrolysis product of ASA. The pharmacological effects of ASA are largely caused by its capacity to acetylate (and inactivate) cyclooxygenase and inhibit prostaglandin synthesis. Methanol is set free from MeS by hydrolysis.

After administration of SA or its esters, the principal metabolite circulating in plasma at comparable concentrations is salicylate. Therefore, RAC considers that in the absence of data for MeS, data from the acetyl ester of SA is acceptable for read across to the methyl ester. Possible effects of the acetyl or methyl moieties generated from acetyl or MeS by hydrolysis are not taken into consideration in such an approach.

Differences in protein binding fractions of salicylate have been described in various species (Kucera & Bullock, 1969). Binding fractions also depend on the experimental conditions, e.g. the salicylate concentration. At a drug level of 150 mg/L, plasma binding fraction were 60 % (human), 58 % (monkey), 55 % (rabbit) and 36 % (rat). Other authors found slightly different binding fractions, however, in all studies binding fractions in humans, primates and rabbits were higher than in rats.

Production and use

MeS is an ingredient used in many fragrance mixtures. It is manufactured in and imported into the European Economic Area in quantities of 1 000-10 000 tonnes per year (ECHA website, 2018). It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Several acute oral toxicity studies in five species (rats, mice, rabbits, guinea pigs, dogs) were available in the CLH report and are summarised in the Table below. The oldest studies were published in the 1950s. Studies according to current guidelines are not available. For most of the studies, detailed information on the experimental conditions is lacking and thus, reliability is poor according to the DS.

***Table**: LD_{50} and resulting classification in different species administered with methyl salicylate.*

Species (n/sex)	Doses (g/kg)	LD_{50}
Rats (5/sex)	n/a	887 mg/kg → Acute Tox. 4
Rats (5/sex/dose)	2.5, 3.15, 3.97, 5.0	3 050 mg/kg (males) 2 640 mg/kg (females) 2 820 mg/kg (combined)
Rats	1.0, 1.25, 1.5, 2.0, 2.25, 2.5, 3.0	1 250 mg/kg → Acute Tox. 4
Rats	n/a	1 220 mg/kg (males) → Acute Tox. 4 1 060 mg/kg (females) → Acute Tox. 4
Mice	n/a	580 mg/kg → Acute Tox. 4
Mice (male)	n/a	1 100 mg/kg → Acute Tox. 4
Mice (male)	1, 1.2, 1.3, 1.5, 1.7	1 390 mg/kg → Acute Tox. 4
Rabbits	n/a	1 300 mg/kg → Acute Tox. 4
Rabbits	n/a	2 800 mg/kg
Rabbits	n/a	2 800 mg/kg
Guinea pigs	n/a	700 mg/kg → Acute Tox. 4
Guinea pigs (male/female)	n/a	1 060 mg/kg → Acute Tox. 4
Dogs	n/a	2 100 mg/kg

n/a = not available

The available LD_{50} values range from 580 mg/kg bw (mice) to doses higher than 2 000 mg/kg bw in rats, rabbits and dogs.

Human data on salicylate poisoning are available due to overdoses of ASA, excessive application of topical agents, ingestion of salicylate containing ointments, use of keratolytic agents or agents containing MeS (e.g. wintergreen oil). In 2004, US poison control centres reported 40 405 human exposures to salicylates, with 12 005 (30 %) cases of MeS. Typical symptoms of salicylate toxicity are hematemesis, tachypnoea, hyperpnoea, dyspnoea, tinnitus, deafness, lethargy, seizures or confusion.

The DS proposed to classify MeS as Acute Tox. 4; H302 with an ATE of 580 mg/kg bw (lowest LD_{50} available).

Comments received during consultation

One MSCA stated that Acute Tox. 4; H302 is justified but preferred an ATE value of 500 mg/kg bw as LD_{50} values of 300 mg/kg bw < Category 4 ≤ 2 000 mg/kg bw lead to a converted ATE value of 500 mg/kg bw.

Another MSCA agreed to the ATE of 580 mg/kg bw.

Assessment and comparison with the classification criteria

Among the 13 studies available, seven reported LD_{50} values lead to a classification as Acute Tox. 4 (300 ≤ LD_{50} ≤ 2 000 mg/kg bw). In the remaining studies, the LD_{50} values reported were above the Guidance value for classification. The lowest LD_{50} value in these studies was reported in a Russian publication to be 580 mg/kg bw in mice, however, it only cited another source without presenting any experimental details. Because the origin of this value remains obscure

and a detailed description of the experimental conditions is not available, this publication is not considered sufficiently reliable to derive the ATE value.

A review published by Lapczynski *et al.* (2007) provides a list of seven acute toxicity studies in three species after oral dosing. LD_{50} values range from 890 to 2 820 mg/kg bw. The lowest LD_{50} value of these studies was published in 1964 (Jenner *et al.*, 1964). An LD_{50} value of 887 mg/kg bw was reported in rats and experimental details are available. RAC notes that this study is considered the key study in the REACH registration dossier (ECHA website, 2019)

RAC concludes that MeS meets the criteria (300 ≤ ATE ≤ 2 000 mg/kg bw) and should be classified as **Acute Tox. 4; H302 (Harmful if swallowed) with an ATE of 890 mg/kg bw**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitising potential of MeS was mainly investigated in several local lymph node assays (LLNA) and guinea pig maximisation tests (GPMT). MeS was applied in concentrations between 1 and 100 %. A concentration dependency was recognised in the LLNA, where concentrations of MeS of > 25 % showed positive results.

The results of the two maximisation assays were negative. However, fewer animals were used than recommended in the OECD test guideline.

Animal data

The DS presented a total set of 12 LLNA. At low concentrations, no relevant lymph node stimulation index (SI < 3) has been observed. Therefore, studies performed with maximum concentrations below 20 % and/or not in compliance with the test guideline, are not presented in this modified table.

Method, guideline	**Species**	**Dose levels**	**Results**
LLNA (similar to OECD TG 429)	Mice	5, 10, 25 % in acetone/olive oil for 3 consecutive days	**Negative** (SI < 3) 5 %: 0.9 10 %: 1.4 25 %: 2.2
LLNA (similar to OECD TG 429)	Mice (CBA/Ca)	10, 20, 25, 50, 100 % (experiment 1) 10, 25, 50 % (experiment 2) 12.5, 25, 50, 100 % (experiment 3) Neat or diluted in (Dimethyl formamide (DMF) or Methylethylketone (MEK) Daily for 3 consecutive days	**Positive** Experiment 1 (DMF) 10 %: 1.2 20 %: 1.6 25 %: 2.4 50 %: 2.6 100 %: 4.0 EC3 = 65 % Experiment 2 (MEK) 10 %: 1.8 25 %: 5.3 50 % 10.7 EC3 = 15 % Experiment 3a (DMF) 12.5 %: 1.5 25 %: 1.7

Method, guideline	Species	Dose levels	Results
			50 %: 5.9 100 %: 7.1 EC3 = 33 % Experiment 3b (MEK) 12.5 %: 2.0 25 %: 2.4 50 %: 7.6 100 % 9.4 EC3 = 28 %
LLNA (similar to OECD TG 429)	Mice (CBA/Ca)	1, 5, 25 % (exp. 1) 5, 10, 25 % (exp. 2) Diluted in DMF or MEK	**Positive** Experiment 1 (DMF) 1.0 %: 1.0 5.0 %: 1.2 25 %: 3 Experiment 2 (MEK) 5.0 %: 2.3 10 %: 2.5 25 %: 7.5
LLNA (deviation from OECD TG 429: injection of 3H-TdR on day 4)	Mice (CBA), male/female, 4/dose	5, 10, 25 % in acetone/olive oil Daily for 3 consecutive days	**Negative** (SI < 3) 5 %: 1.3 10 %: 1.0 25 %: 0.8
LLNA (similar to OECD TG 429)	Mice (BALB/c)	20, 40, 80 % in 4:1 acetone/olive oil daily for 3 consecutive days	**Positive** EC3 = 48.15 %, No excessive local irritation

Human data

Several human data are available, including 3 human volunteer induction studies, 8 diagnostic studies and 2 case reports.

Report	Test substance	Relevant information about the study	Observations
Induction studies			
Human maximisation with 25 healthy volunteers	12 % wintergreen oil (containing 80-99 % MeS)	Application of 12 % wintergreen oil in petroleum under occlusion for 5-alternate-day 48 h period after pre-treatment of patch site for 24 h with 5 % aqueous SLS under occlusion. After 10 to 14 day rest period, 5 % SLS was applied under occlusion for 30 min on the left side of the back prior to challenge patch of MeS under occlusion for 48 h on the right side.	0/25 positive reactions
Human maximisation with 27 healthy volunteers	8 % MeS	Application of 8 % MeS in petroleum under occlusion for 5-alternate-day 48 h periods after pre-treatment with 5 % aqueous SLS under occlusion.	0/27 positive reactions

Report	Test substance	Relevant information about the study	Observations
		After a 10 to 14 days rest period, 10 % aqueous SLS solution under occlusion was applied prior to challenge consisting on a 48 h patch of MeS under occlusion. Reactions were read at patch removal and 24 h later.	
Human repeated insult patch test (HRIPT) with 13 males and 26 females	1.25 % MeS	Nine applications of 1.25 % MeS over a 3 week-period. 24 h challenge patch test on the 6th week. Reactions were scored at 24 and 72 h after patch removal	0/39 positive reactions
Diagnostic studies			
Patch test in 4 600 patients - 2 784 patients with contact dermatitis - 189 patients with dermatitis of hands - 135 patients with photoallergy - 1 491 healthy patients	2 % MeS in petrolatum	**Unselected patients** A total of 4 600 patients were patch tested in the 5-year period 1973-1977 in the Allergy Department of Barcelona University, Spain. Patch test with ICDRG (International Contact Dermatitis Research Group) series including 2 % MeS in petrolatum	0.13 % (6/4 600) positive reactions It is not specified in which group of patients the positive results were found
Patch test in 183 patients	2 % MeS	**Selected patients** Patch test of the North American Contact Dermatitis Group from 1 July 1975 to 30 June 1976. A1 Test® strips or Finn Chambers® were used. Tests were read at 48 and 96 h.	1.6 % positive reactions
Patch test in 241 patients (61 males, 180 females)	2 % MeS in PMF (yellow soft parafin)	**Selected patients** Patch tests from October 1981 and June 1983 in Scotland. Perfume screening series including MeS.	1.2 % positive reactions = 3 females with grade 2 (definite erythema) and above
Patch test in 585 eczema patients	2 % MeS in petroleum	**Selected patients** Standard patch tests on eczema patients in North America. 2 periods: 1978-1979 with 301 patients 1979-1980 with 284 patients	1 % positive reaction for the period 1978-1979 2 % positive reactions for the period 1979-1980

Report	Test substance	Relevant information about the study	Observations
Patch test in 89 patients: - 19 with eyelid dermatitis - 70 with dermatitis at other sites	1 % MeS in petroleum	**Selected patients** Patch tests between January 1980 and May 1987 in the Contact Dermatitis Clinic of St Michael's Hospital in North America. A1 Test® strips or Finn Chambers® secured with Scanpor tape for a period of 48-72 h. Reactions read after removal and re-examined 48 or 72 h after the first reading. Responses scored as 1+, 2+ or 3+ were determined to be positive; doubtful responses were scored as negative. Irritant responses were scored as negative.	0 % positive reaction among the 19 patients with eyelid dermatitis 1.4 % positive reactions among the 70 patients with dermatitis at other sites
1 825 patients	2 % MeS in petroleum	**Unselected patients** Multicenter study conducted from September 1998 to April 1999. Test procedures were carried out according to internationally accepted criteria. Potential irritancy was excluded in a pilot study involving 200 patients.	0.4 % positive reactions (7/1 825)
Patch test in patients - with photosensitivity dermatitis with actinic reticuloid syndrome (50) - with polymorphic eruption (32) - with contact dermatitis (457)	2 % MeS in PMF	**Selected patients** A1 Test® strips for 48 h. Any reactions read at patch removal, and at 72 h after the application.	2 % (1/50) positive reactions in patients with photosensitivity dermatitis with actinic reticuloid syndrome 0 % positive reaction in the two other groups (0/489)
Work place study			
Patch test in 267 health care employees with contact dermatitis (82 males and 194 females)	2 % MeS in petroleum	**Epidemiological study with selected workers** Patch test among health care employees in Italian hospital. GIRDCA standard series (Gruppo Italiano Ricerca Dermatiti da Contatto ed Ambientali), "health" series and, when necessary, a "rubber" series. Patches removed after 2 days. Reading on days 2 and 3.	0 % positive reactions (0/276)
Case reports			
Case report	2 % MeS in olive oil	A 79 year-old woman had a rectangular pruritic erythematous	1 case Patch test

Report	Test substance	Relevant information about the study	Observations
		macule on the hip following the use of a compress containing MeS.	positive to MeS on day 2 (+) and day 3 (+)
Case report	2 % MeS in arachis oil	A 63 year-old Iraqi businessman developed an acute dermatitis of the neck, upper back, shoulders and dorsa of the hands after applying an analgesic ointment.	1 case Patch test positive to MeS (grade 2 at 48 h)

Animal data

The substance was negative in both Guinea pig maximisation assays. However, fewer animals than recommended in the OECD test guideline were used. This might decrease the sensitivity of the test for substances with low sensitising potential.

Regarding the 12 LLNA studies, most summarise the results of sets of chemicals, including MeS, during the validation of the LLNA as a regulatory test protocol. MeS was negative at concentrations up to 20 % or 25 %, while at higher concentrations positive results were found in the majority of experiments following the guideline protocol. Some studies deviate from the guideline protocol, e.g. using the rat instead of mice.

Human data

In the three human volunteer induction studies (2 maximisation studies and one (human repeated insult patch test – HRIPT study) no signs of sensitisation to MeS was reported. The number of volunteers ranged from 25 to 39 with concentrations ranging from 1.25 to 8 % MeS or 12 % wintergreen oil, containing 80 to 99 % MeS. These studies lack detailed information as the cited reference is a review article.

Of the 8 diagnostic patch testing studies, 7 provide positive results. A distinction must be made between patch testing "unselected/consecutive" patients, i.e. all patients who are patch tested for suspected contact sensitisation, and "aimed/selected" patch testing, i.e. application of allergens only in the subset of patients in whom exposure to the particular allergens of the applied "special series" is suspected. In general, the latter "aimed" approach will usually yield higher sensitisation prevalence than the testing of not-further-selected "consecutive" patients. Thus, information on the inclusion of an allergen either in a baseline series (tested in virtually all patients) or in a special series (applied in an aimed fashion) must be considered. Among the diagnostic studies available with MeS, there were 2 studies with unselected patients and 6 with selected patients. The concentrations used ranged from 1 to 2 % MeS. Diagnostic studies with unselected patients included 1 825 or 4 600 patients and showed a frequency of positive reactions of 0.13 % or 0.4 % respectively. Diagnostic studies with selected patients included 19 to 585 patients and report a frequency of positive reactions between 0 and 2 %.

Finally, two case reports with positive results of skin sensitisation after exposure to 2 % MeS in olive or arachis oil were reported.

In summary, MeS has shown to be a skin sensitiser in diagnostic studies with an incidence < 1 % in unselected patients and ≤ 2 % in selected patients.

The DS proposed that the positive reactions should be considered as sensitising effects, and MeS should be classified as Skin Sens. 1B.

Comments received during consultation

Two downstream users were of the opinion that MeS should not be classified as a skin sensitiser as there are studies indicating that MeS is not a skin sensitiser, but at concentrations above 25 % may induce false positive results due to its irritation properties. They claimed that there is no indication for a skin sensitisation concern from the use of MeS when used for local pharmaceutical treatment at concentrations up to 10 % based on pharmacovigilance data. Additionally, assessment of MeS in guinea pig maximisation assays using optimal conditions for maximal stimulation of the skin immune system did not result in skin sensitisation.

Similarly, one manufacturer and one importer concluded that MeS should not be classified as a skin sensitiser, as the cellular proliferation effects observed in the local lymph node are likely to be an effect of the irritation properties and not an indication for skin sensitisation. In addition, the human data also does not reveal a clear indication that the substance is a skin sensitiser.

Two MSCAs supported the proposal to classify MeS as a skin sensitiser 1B; H317.

Assessment and comparison with the classification criteria

Non-human data

Three types of animal tests can be used directly for classification purpose according to CLP: LLNA, GPMT and Buehler assay. The criteria for LLNA are as follows:

1A: EC3 value ≤ 2 %

1B: EC3 value > 2 %

In an LLNA using four concentrations of MeS (vehicle: MEK, methylethylketone) the following results for the stimulation index and EC3 value were obtained:

12.5 %: 2.0; 25 %: 2.4; 50 %: 7.6; 100 % 9.4; EC3 = 28 %.

Corresponding results were obtained in a parallel experiment under identical conditions, but with DMF as a vehicle:

12.5 %: 1.5; 25 %: 1.7; 50 %: 5.9; 100 %: 7.1; EC3 = 33 %

Since these guideline-conforming LLNA assays in female CBA mice yielded EC3 values > 2 %, RAC is of the opinion that MeS fulfils criteria for classification as Skin Sens. 1B.

Human data

According to CLP, the frequency of occurrence of skin sensitisation should be considered as a first step to conclude on classification for skin sensitisation. Data show that only low to moderate frequency of skin sensitisation is found in selected patients. These tests represent about 30 cases with positive patch test reactions. In addition, two case reports are available.

Overall, based on animal data, MeS fulfils criteria for classification Skin Sens. 1B. Based on human data, MeS also fulfils criteria for classification Skin Sens. 1B.

RAC agrees with the DS that MeS should be classified as **Skin Sens. 1B; H317 (May cause an allergic skin reaction)**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility and reproductive function

The DS presented a number of studies conducted to investigate adverse effects on sexual function and fertility or on development after exposure to MeS. Fertility studies reported by the DS cover an extensive time period that starts in the 1960s and extends to the early 2000s. According to the DS, none of the studies reported any significant and/or consistent effect on fertility. Therefore, the DS was of the opinion that no classification is justified for MeS for adverse effects on sexual function and fertility.

Table 17 of the CLH report provides an overview of the animal studies used for assessing the classification of MeS regarding reproductive toxicity. They are briefly summarised in the background document under "Supplemental information – In depth analyses by RAC".

Effects on Development

In addition to the reproductive toxicity studies presented above, the DS evaluated several developmental toxicity studies in rats and rabbits summarised below.

Two studies are available in rabbits and rats focussing on the period of organogenesis. In both studies, MeS was injected subcutaneously.

In the rabbits study there was no treatment related effect on the numbers of corpora lutea, implants or live foetuses, dead embryo / foetus indices or body weight of live foetuses. There was no placental anomaly, no external, visceral or skeletal anomalies related to MeS treatment.

In rat study, no mortality or clinical signs occurred in the treated groups. Statistically significant depression of body weight (< 5 %), bw gain (≥ 10 %) and food consumption was reported in dams at 200 mg/kg bw/d. There was no effect of the treatment on the number of corpora lutea, implants, live and dead foetuses, sex ratio or placental anomalies. External anomalies, characterised principally by craniorachischisis and gastroschisis were detected at levels above HCD (2.86 % versus 0.01 %). but not considered to be statistically significant. Visceral anomalies were also increased but considered to be not statistically significant. Skeletal variations were also observed to have a statistically significant increase at the highest dose and in addition, there was a delay of ossification of the vertebrae, sternebra, metacarpus, metatarsus and phalanges.

In another study, MeS was given subcutaneously to pregnant and lactating rats. There was a statistically significantly lower mean body weight and bw gain during gestation at the top dose of 200 mg/kg bw/d with significantly decreased food consumption during gestation and lactation. In male offspring, a statistically significant decrease in the birth index (-6 %) index (-6 %) and a lower body weight (-9.2 %) were observed in live newborns in the top dose group with a trend toward a decrease in the number of litter and live newborns and a trend toward an increase in the stillbirth index. These effects were considered attributable to MeS.

Other effects, such as, statistically significant decrease in the differentiation indices of incisor eruption in both sexes, skeletal anomalies, cleavage of the balanopreputial gland and statistically significant changes in the weights of organs (brain, lungs, testes, ovaries, kidneys) were observed in the top dose group.

There are also several studies with some shortcomings and unusual routes of administration, such as dermal application or intraperitoneal injection, where the effects varied from severe toxicity and 100 % resorption (Infurna *et al.*, 1990 – only abstract available) to neural tube defects (Overman & White 1983) and lethality, external malformations, visceral and skeletal anomalies and effects on differentiation indices (Kavlock *et al.*, 1982; Daston *et al.,* 1988). The

lowest developmental NOAEL are < 60 mg/kg bw/d in rats exposed subcutaneously from GD6 to LD21 (FDA, 2006b) and 75 mg/kg bw/d in a 3-generation study in rats by oral route (Collins *et al.*, 1971).

Human data

The DS did not present any human data on MeS. However, because human data are available for another salicylate ester, ASA, the DS presents several – mostly retrospective – publications with aspirin during pregnancy.

It is difficult or even impossible to estimate the causal relationship between the effects observed in retrospective studies and the salicylate exposure, because the drug had been taken for certain diseases – such as fever or viral infections – which might pose a risk to pregnancy on their own.

The studies by Richards (1969), Nelson & Forfar (1971), Lynberg *et al.* (1994), Kozer *et al.* (2002) reported defects on central nervous system, alimentary tract, talipes, achondroplasia, anencephaly, spina bifida and encephalocele and other congenital malformations.

A large multi-site population-based case control study was carried out by Hernandez *et al.* (2012). Significant association was found between aspirin consumption and anencephaly/craniorachischisis (total and isolated), anophthalmia/microphthalmia (total), cleft palate (total and isolated) and amniotic bands/limb body wall (total and isolated).

In conclusion, even if most of the epidemiological studies with ASA do not report an increased risk of adverse effect on development at therapeutic dosage, there are some indications of foetal lethality and malformations with this compound. However, due to limitations of the retrospective studies, such as misclassification of exposure, confounding factors and lack of quantitative data, human data are considered inadequate to firmly conclude on the developmental toxicity of salicylates by the DS.

Comments received during consultation

Three downstream users considered that MeS should not be classified as Repr. 1B as some studies were conducted by the subcutaneous route. According to them, findings at 200 mg/kg (FDA, 2006c) occurred at maternally toxic doses and the rabbit study did not show any potential for reprotoxic effects when MeS was given during the period of organogenesis (GD6-18). They also argued that the hamster study was performed at doses well above the human toxic level and salicylic acid, as the relevant metabolite, was evaluated by RAC as Repr. 2.

One downstream user, two manufacturers and one importer stated that Repr. 1B is not justified. Another downstream user proposed to classify MeS as Repr. 2; H361d based on the RAC opinion on SA in 2016.

One MSCA supported the classification for developmental effects as Repr. 1B.

Another MSCA proposed to discuss if classification as Repr. 2; H361 or Repr. 1B is appropriate, because the available animal and toxicokinetic data delivered clear evidence of developmental effects, independently of the route of administration. The similarity of teratogenic effects of MeS and SA, the lack of human data on MeS, the generation of methanol as an additional hydrolysis product and the toxicokinetic differences (possibility of distribution of intact parent MeS into target tissues and toxic action of either parent and/or hydrolysis products of SA and methanol in the target tissue) point to different and possibly additional effects of MeS in humans when compared to SA. In case the same classification strategy is applied as for SA, MeS would need to be classified as Repr. 2; H361d. In case not, a harmonised classification as Repr. 1B; H360D has to be discussed.

Assessment and comparison with the classification criteria

Fertility and reproductive function

Seven different studies were performed to investigate the effects of MeS on fertility. No statistically significant effects on fertility and mating were reported in rats at doses up to 250 mg/kg bw/d by oral route and 300 mg/kg bw/d by subcutaneous application and in mice at doses up to 750 mg/kg bw/d which were the highest doses tested. Even if most of the fertility studies show a number of deficiencies compared to OECD test guidelines in term of parameters studied, none reported any significant and/or consistent effect on fertility.

Human data are conflicting and do not allow to draw a clear conclusion.

There is insufficient evidence that MeS exhibits adverse effects on sexual function and fertility.

RAC concurs with the proposal by the DS that no classification is justified for MeS for adverse effects on sexual function and fertility.

Development

With respect to developmental toxicity, RAC is of the opinion that MeS should be classified in Cat. 2, mainly due to the weight of evidence put on the human data with ASA which do not indicate that ASA is a human teratogen.

Among the salicylates, the vast majority of human data derive from the use of ASA in pregnant women. The drug is widely used as an analgesic, antipyretic and anti-inflammatory agent. Some older retrospective studies reported malformations in children from women treated during pregnancy with aspirin for viral infections, fever and other indications.

Larger, prospective studies did not show a teratogenic effect of aspirin and, for women at risk for pre-eclampsia, the drug shows some benefit when given during pregnancy.

In a cohort of 50 282 gravidas and their offspring in the U.S.A., malformation rates were similar in the children of 35 418 women not exposed to aspirin, 9 736 with intermediate exposure, and 5 128 women heavily exposed during the first four lunar months of pregnancy. After controlling a wide range of potential confounding factors using multi-variate analysis, the observed and expected numbers for a variety of malformation categories were similar in all three comparison groups. The data suggest that aspirin is not teratogenic (Slone *et al.*, 1976).

Nowadays, professional associations recommend the prophylactic daily use of low-dose aspirin in pregnant women who are considered to be at high risk for pre-eclampsia. More than 30 trials have investigated the benefit of ASA at doses of 50 to 150 mg per day for the prevention of pre-eclampsia. These studies showed that such therapy resulted in a 10 % lower incidence of pre-eclampsia. A recent trial showed that among women who were at high risk for preterm pre-eclampsia, the administration of ASA at a dose of 150 mg per day from 11 to 14 weeks of gestation until 36 weeks of gestation resulted in a significantly lower incidence of preterm pre-eclampsia in comparison to placebo. No significant between-group differences in the incidence of neonatal adverse outcomes or other adverse events were observed between women treated with ASA or placebo (Rolnik *et al.*, 2017).

Salicylic acid has been classified by RAC in Category 2 for developmental toxicity in March 2016. In a weight of evidence approach, this recommendation was mainly based on the lack of birth defects in humans, despite clear teratogenicity in rats and monkeys.

The Committee considers the following quotes from the RAC (2016) opinion on SA to be relevant for MeS:

"*Neither ASA nor SA are proven human developmental toxicants. There is a lack of evidence to support an increased risk of birth defects following exposure to ASA. Also, the evidence for other developmental effects has uncertainties. Taking that into account, classification in Category 1A is not justified.*

In the study of Wilson et al. (1977), when general embryotoxicity of rats and monkeys to ASA was compared at equivalent dosages, some differences were detected. According to the study author this difference in effects seen can be attributable to the differences in embryonic exposure; since the free (unbound) SA is responsible for the teratogenic potential and the binding capacity differs between species, the rat embryo is exposed to higher levels and for a longer duration than the monkey embryo.

In rats plasma concentrations of salicylate 20 minutes after oral administration of methyl- or acetylsalicylate at a dose of 500 mg/kg bw were 217 ± 16.1 mg/L (MeS) and 209 ± 18.6 (ASA) and 60 minutes after dosing salicylate concentrations of 278 ± 16.7 mg/L (MeS) and 274 ± 23.5 (ASA) mg/L were measured (Davison et al., 1961) indicating a similar toxicokinetic behaviour of both esters in rats.

In humans, no malformations could be detected; based on the assumption of a similar teratogenic potency in all species, a hypothetical human threshold for malformations around of 200 mg/L of total salicylate in maternal serum was calculated".

RAC is of the view that, with MeS, the situation is similar to SA and it is a matter of consistency to classify the methylester of SA accordingly.

Several studies showed that MeS is teratogenic in rats, but not in rabbits. This finding is in agreement with salicylic acid, which causes a similar pattern of neural tube defects and other malformations in rats and monkeys, but not in rabbits.

Malformations observed with MeS via the subcutaneous route occurred at low incidence at the highest dose only, which caused significant maternal toxicity". In comparison to concurrent controls, increased incidence in foetuses of severe neural tube defects, such as craniorachischisis, was not statistically significantly different. At the top dose, incidence of skeletal variations was significantly increased, which can be interpreted as a consequence of maternal toxicity and not substance-related.

Based on the weight of the evidence, RAC is of the opinion that MeS should be classified as **Repr. 2; H361d (Suspected of damaging the unborn child)** based on positive animal experiments with MeS and negative human data with acetylic salicylic acid.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to classify the substance as Aquatic Chronic 3; H412. The lowest L(E)C_{50} obtained in acute aquatic toxicity studies was 1.6 mg/L for the algae *Desmodesmus subspicatus*. This value was above the classification threshold value of 1 mg/L and no acute classification was warranted. The substance was considered rapidly degradable based on a weight of evidence approach. Based on the log K_{OW} < 3, MeS had a low potential for bioaccumulation. The lowest NOEC value was 0.79 mg/L for algae which for a rapidly degradable substance was the basis for the proposed Aquatic Chronic 3; H412 classification.

Degradation

There was one non-GLP ready biodegradability study available performed according to a draft Ecotoxicology Section Standard Operating Procedure No 158 01, which is based on OECD TG 301B. The test showed 98.4 % degradation after 28 days with a test substance concentration of 10 mg/L. There were no data on the toxicity control and the raw data for the blank and test samples are not available. Due to the lack of data, this study was only used as supportive information. A second non guideline study focused on the ability of a microbial mixture (five *Pseudomonas*, one *Klebsiella*, four *Rhodococci* and two fungal strains) to degrade a representative sample of methylated and chloro-methylated compounds. MeS at a concentration of 200 mg/L degraded 100 % in 7 days. The inoculum used did not correspond to recommendations for ready biodegradability testing. Additionally, only primary biodegradation was measured and not ultimate biodegradation. This study was used only as supportive information. A screening-level hazard characterisation made on benzyl derivatives category showed the ready biodegradability for all members of the group, which include MeS. Furthermore, the QSAR predictions with BIOWIN 4.10 indicated that all 2-hydroxybenzoate esters subcategory III from the US EPA report (among which MeS belongs) were readily biodegradable substances. Consequently, the DS applied a weight of evidence approach for considering MeS as a rapidly degradable substance.

Bioaccumulation

MeS had a low potential for bioaccumulation based on the experimental log K_{OW} = 2.55, which was below the cut-off value of 4 for bioaccumulation. The purity of the test substance was not given. The REACH Registration Dossier (full, 15/08/2019) indicates that the value originates from the databank of Sangster (1989), which was reported as a key reference in the ECHA IR & CSR Guidance Document. The databank contains experimental log K_{OW} data, retrieved from the literature, on over 20 000 organic compounds. For each compound, whenever possible, the compiler gives the log K_{OW} value which, in their judgment, is closest to the true value. For MeS, this databank reports 5 experimental log K_{OW} values ranging from 2.08 to 2.98. The recommended value of 2.55 is selected as key value for the assessment of the substance.

Aquatic toxicity

Table*: Reliable aquatic toxicity data on MeS, ethyl salicylate and sodium salicylate and 2-salicylic acid*

Test substance	Method	Species	Endpoint	Exposure	Results mg/L	Reference
Fish						
Ethyl salicylate	equivalent or similar to OECD TG 203, not GLP	*Pimephales promelas*	Mortality	96 h flow-through	LC_{50}: 19.8 mm (*	Geiger *et al.*, 1985a
Sodium salicylate	equivalent or similar to OECD TG 203, not GLP	*Pimephales promelas*	Mortality	96 h flow-through	LC_{50}:1 370 mm	Geiger *et al.*, 1985b
Invertebrates						
Ethyl salicylate	OECD TG 202, GLP	*Daphnia magna*	Mobility	48 h static	EC_{50}: 28 (im, DOC analysis) (**	Noak, 2001
2-salicylic acid	equivalent or similar to OECD TG 202, not GLP	*Daphnia magna*	Mobility	48 h static	EC_{50}: 870 Nominal concentration, no analytical monitoring	Kamaya *et al.*, 2005

Algae						
MeS	OECD TG 201, GLP	*Desmodesmus subspicatus*	Growth rate	72 h static, closed system	ErC_{50}: 1.6 (1 NOEC: 0.79 (1 ErC_{50}: 1.475 (2 NOEC: 0.79 (2 ECr_{10}: 1.033 (2 geometric mm	Vryenhoef and Mullee, 2010

mm = mean measured concentrations
im= initial measured concentrations
(*the stability of the substance cannot be estimated in regard to nominal concentration that were not mentioned in the publication. But regarding measured concentration, it is clear that in every replicate considered separately the concentration of test substance was satisfactorily maintained during the test. Therefore, the DS considered the validity criteria fulfilled. Mean measured concentrations corrected for percent recovery: 2.73, 4.82, 7.70, 14.9 and 26.2 mg/L. It should be noted that samples were not taken at 96 h (the mean measured concentration was calculated from measurements at t0, 24, 48 and 72 h)
(** test concentrations have only been measured at 0 h
(1 Geometric mean values calculated using data on 0 and 72 h
(2 Information from PC comments, geometric mean values calculated using data on 0, 49 and 72 h

Basis for read-across

The main assumption to justify the read-across approach is that methyl and ethyl salicylate have a similar chemical structure. Both substances are 2-hydroxybenzoate, methyl ester and ethyl ester, respectively. Therefore, both substances have the same functional groups in their chemical structure, and the addition of an alkyl group "-CH_2-" in the ester function for ethyl salicylate compared to MeS was not expected to have a significant impact on the biological and physico-chemical properties of the substance.

This assumption is supported by the very similar physico-chemical properties of the substances, including water solubility and vapour pressure. The log K_{OW} value of ethyl salicylate was slightly higher than the one for MeS (i.e. 3.09 and 2.55 respectively). Therefore, it could be expected that ethyl salicylate has higher effects on biological cells than MeS, and application of the read-across approach represent a worst case scenario.

To support the assumption that MeS is less toxic than ethyl salicylate, data on SA was used to show that the 2–hydroxybenzoic acid is less toxic than the methyl ester, and therefore that the toxicity of the substituted 2-hydroxybenzoic form is proportional to the length of the substituent.

Acute Aquatic toxicity

Fish

The only acute fish toxicity test with MeS was not reliable because of uncertainty regarding the test substance concentration during the test. The DS used data on the analogous substances ethyl salicylate and SA in a weight of evidence approach. The tests followed a method similar to OECD TG 203. The validity criteria were fulfilled in the test with ethyl salicylate where the measured concentrations were 0 (control), 2.73, 4.82, 7.70, 14.9 and 26.2 mg/L. At 96 h, no mortality was observed at 14.9 mg/L and 100 % of fish exposed to 26.2 mg/L died. The resulting approximate LC_{50} (96 h) was 19.8 mg/L, based on mean measured concentrations. The aquatic toxicity of SA was assessed based on its sodium salt to avoid the pH effect. In the acute toxicity study with *Pimephales promelas*, fish were exposed to SA sodium salt at average measured concentrations of 0, < 50, 497, 536, 837, 867, 1 238, 1 272, 2 211, 2 217, 3 442 and 3 573 mg/L. The LC_{50} (96 h) was 1 370 mg/L based on mean measured concentrations.

In conclusion, the result obtained with ethyl salicylate was used in a worst-case read-across approach to assess the fish toxicity of MeS.

Invertebrates

There was no reliable acute toxicity study available on MeS. Therefore, similar to the assessment of acute toxicity to fish, a weight of evidence approach was applied for the assessment of the toxicity to aquatic invertebrates of MeS. Ethyl salicylate and SA were used as analogous substances. One reliable key study is available for ethyl salicylate for this endpoint. In this acute *D. magna* toxicity study, the acute immobilization of the test item was determined according to OECD TG 202 following GLP. The initial test concentrations were determined based on DOC analysis: 9.2, 19, 40, 84 and 165 mg/L. Exposure of daphnids to ethyl salicylate resulted in a 48 h EC_{50} value of 28 mg/L (initially measured concentration).

The 48 h acute toxicity study of SA to *D. magna* was conducted under static conditions with nominal concentrations from 276 to 2 210 mg/L. The 48 h EC_{50} was determined to be 870 mg/L.

In conclusion, the result obtained with ethyl salicylate is used in a worst-case to assess the toxicity to aquatic invertebrates of MeS.

Algae and aquatic plants

There was one reliable study available for acute algae toxicity using MeS. Its effect on the growth of the freshwater green algae *Desmodesmus subspicatus* was investigated in a 72 h static test according to OECD TG 201 and GLP compliant.

Following a preliminary range-finding test, *Desmodesmus subspicatus* was exposed at concentrations of 0 (control) 6.25, 12.5, 25, 50 and 100 mg/L. The initial concentration were analytically confirmed, however at 72 hours a concentration dependent decline in measured concentrations was observed. Thus, geometric mean measured concentrations were used for calculating EC_{50} values. The 72 h EC_{50} was 1.6 mg/L for growth rate.

Chronic aquatic toxicity

There was no chronic toxicity data available for fish and invertebrates. The 72 h NOEC for growth rate and biomass of 0.79 mg/L resulted from the only algae test available.

Comments received during consultation

Comments were received from one MSCA and from a leading group of REACH registrants.

The registrants pointed out that the available OECD TG 201 algae study had deficiencies and should not be used for classification. They submitted an assessment of the study report focusing on paragraphs 37 and 40 of OECD TG 201. According to the assessment, the current OECD TG 201 study did not allow a final assessment of chronic toxicity in algae due to failures in the methodology mainly regarding analytical measurement. Moreover, the EC_{10} for growth rate calculated in the assessment was > 1 mg/L indicating that the study most likely overestimates the effects of MeS. More details of the assessment report are described under the Additional Key Elements. The DS answered that they did not include time points at 24- and 48 h following the REACH Guide R7b p. 75: "*For static tests, where the concentrations do not remain within 80-120 % of nominal, the effect concentrations should be expressed relative to the geometric mean of the measured concentrations at the start and end of the test*." They considered that the study should be used for classification. The commenting MSCA agreed with the DS.

The MSCA paid attention to the available fate data. The ready biodegradation test is a non-GLP test with limited reliability due to lack of raw data. In addition, the reported Henry's law constant of 4.76 $Pa \cdot m^3/mol$ indicates the test item may be lost from the aquatic phase meaning the test method may not be the most appropriate. Additional supporting fate data involving non-standard methods and/or inoculum and unknown sample composition were included. While QSAR data

were quoted, it is unclear if the test item fell within the model domain and if the QSARs were valid. Additional information would be welcomed to support MeS as rapidly degradable e.g. read-across with reliable analogue data and/or valid QSARs. Without this information a substance would normally be considered not rapidly degradable. In the answer to the consultation comments, the DS presented QSAR calculations made with EPISUITE and the Danish QSAR Database, which showed that MeS was readily biodegradable while being in the applicability domain of each model.

The MSCA also asked if there was analytical verification at study termination in the ethyl salicylate *D. magna* test giving a 48 h EC_{50} of 28 mg/L based on initial measured concentrations. The DS answered that the study was considered of reliability 2. The test item was only analytically verified at the beginning of the test. However, the test item instability during experiment was specifically reported for the algae study with MeS and not in the other assays. The DS was of the view that the stability of ethyl salicylate in other experiments confirmed that the EC_{50} (48 h) value of 28 mg/L for *D. magna* is acceptable and the instability of the test item was specific to the algae study.

The MSCA also thought that it is unclear if algae were the most chronically sensitive trophic level. They welcomed additional toxicity data for relevant analogues with a clear read-across justification. The DS informed that no additional chronic aquatic toxicity data on the level was available on analogues. Classification based on one toxicity data for one trophic level is allowed. In addition, the DS presented QSAR data from the Danish QSAR database where the acute toxicity for the 3 trophic levels are in the same order of magnitude. According to the DS, MeS was included in the applicability domains.

Algae toxicity, assessment of the original study

The assessment report concerns interpretation of the biological results of Harlan Study No 1975/0003, Algae Growth Inhibition Test with MeS (CAS: 119-36-8) and re-evaluation of EC_X-values and concentration effect curves. Selected details of the report are presented in the BD.

<u>Decline of Test Item Concentrations</u>

During the main test, a strong decline of test item concentrations was observed. From a stability pre-experiment, the most important cause of the substance degradation was identified as absorption/metabolism by the algae cells.

<u>Inhibitory Effect on Algae Growth</u>

In the two lowest concentration a decrease of algae growth inhibition at 72 h was observed which could be explained by the degradation of the test item (measured concentration at 72 h were 0, 1.1, 1.5, 2.2, 50 mg/L). However, this conclusion is partly compromised by the lack of a clear concentration effect relationship at 49 hours, which cannot be explained from a biological point of view. The inhibitions at 49 h were 0, -80.6, 62.7, 39.2, 150, 98.2 % for nominal concentrations of 0, 6.25, 12.5, 25, 50, 100 mg/L, respectively. Therefore, as no analytical results are available for 25 and 49 hours, an assessment of the decrease of inhibition is not possible for these test concentrations.

<u>Decline of Test Item Concentrations</u>

During the main test, a strong decline of test item concentrations was observed. The test item concentrations measured at the start of test confirmed the correct preparation of the test media. At the end of the test, the test item concentrations were below LOQ at the lower test concentrations and were 24 mg/L at the highest test concentration.
Stability pre-experiments were performed (before and after the main test) for better understanding of the behaviour of the test item in test water.

From the results of these tests, it is concluded that the decrease of the test item concentrations could be caused by hydrolysis (small part), photolysis (more likely) and absorption/metabolism by the algae cells (very likely). Furthermore, adsorption on the surfaces of the test vessels are indicated although the specific properties of the molecule make this unlikely.

Conclusions

In summary, due to the degradation of the test item and the decrease of inhibition of algae growth, the geometric mean concentrations should be used. However, as no analytical measurements were performed at 25 and 49 hours, the decline of test item concentrations is not well documented which affects the calculated endpoints values. In addition, a precise comparison of the decline of test concentrations with the decrease of algae growth inhibition is not possible. Furthermore, a clear interpretation of the biological results is partly compromised by the lack of a concentration effect relationship after 49 hours. This cannot be explained from a biological point of view and is in contrast to the results after 25 and 72 hours where a clear concentration effect relationship was observed.

Aquatic toxicity

RAC has made estimations with ECOSAR v.1.11 to complement the very scarce dataset on aquatic toxicity on MeS. The estimated data and the test data provided by the DS are presented in the table below.

***Table**: Data available for evaluation of aquatic toxicity of MeS*

	MeS [1]				Ethyl salicylate [2]			
	ECOSAR Class Esters	ECOSAR Class Phenols	ECOSAR Baseline toxicity		ECOSAR Class Esters	ECOSAR Class Phenols	ECOSAR Baseline toxicity	
	Predicted mg/L			Data mg/L	Predicted mg/L			Data mg/L
Fish, 96 h LC_{50}	9.034	9.189	35.874		5.098	4.249	14.191	19.8 [3] mm [*]
Daphnids 48 h LC_{50}	17.604	3.620	21.537		9.425	2.083	8.915	EC_{50}: 28 [3] (im) [**]
Green algae 96 h EC_{50}	6.809	15.702	20.201	72 h E_rC_{50}: 1.6 [3] 1.475 [5] mm	3.372	8.614	10.086	72 h E_rC_{50}: 9.47 (im) [4]
Fish ChV	0.599	1.013	3.745		0.305	0.504	1.563	
Daphnids ChV	10.118	0.688	2.453		4.704	0.396	1.152	
Green algae ChV	2.123	7.320	5.989	72 h NOEC: 0.79 [3] EC_{10}: 1.033 [5] (mm)	1.233	3.991	3.308	72 h EC_{10}: 7.89 (im) [4]
Fish (SW) 96 h LC_{50}	13.244	3.703	-		7.252	1.564	-	
Mysid 96 h LC_{50}	9.675	-	-		4.159	-	-	
Fish (SW) ChV	2.116	-	-		1.266	-	-	
Mysid (SW) ChV	208.065	-	-		32.875	-	-	

[1] Log K_{OW} 2.604, water solubility 700 mg/L
[2] Log K_{OW} 3.095, water solubility 3 317 mg/L
[3] From the CLH Report
[4] REACH Registration
[5] Consultation comments

(im) = measured initial concentration
(mm) = mean measured concentration
(*the stability of the substance cannot be estimated in regard to nominal concentration that were not mentioned in the publication. But in regard to measured concentration, it is clear that in every replicate considered separately the concentration of test substance was satisfactorily maintained during the test. Therefore, the DS considers the validity criteria fulfilled.
(** test concentrations have only been measured at 0 h
ChV = The ChV, or Chronic Value, is defined as the geometric mean of the NOEC and LOEC.

Assessment and comparison with the classification criteria

Degradation

In a ready biodegradability study performed according to OECD TG 301B with adaptations for volatile substances (sealed vessel), the biodegradation was 98.4 % after 28 days. The 10-day window criteria were fulfilled. Consequently, MeS was considered readily biodegradable. The study report lacked information needed for validity checking e.g. information on replicates and CO_2 evolution in the inoculum blank at the end of the test. The study report by King from 1993, The Biodegradability of Perfume Ingredients in the Sealed Vessel Test, refers to study report published in Chemosphere, Vol. 23, No 4, pp. 507-524 in 1991 for development and validation of the method used. This publication by Birch and Fletcher is titled The Application of Dissolved Inorganic Carbon Measurements to the Study of Aerobic Biodegradability. The article was about developing a test that is essentially the same as the Sturm CO_2 Production tests (OECD TG) but with greater simplicity of the technique and the high precision of the data. It did not include any validity criteria as such. This study has been referenced and used as the basis of OECD TG 310.

While the ready biodegradability study report does not contain all information needed for checking its validity, on the other hand, the referenced publication strengthens the case. Considering the supporting data provided by the DS together with the BIOWIN v.4.10 estimation, RAC considers MeS as rapidly degradable for classification.

Bioaccumulation

RAC agrees with the DS that MeS has a low potential for bioaccumulation based on the experimental log K_{OW} = 2.55. The KOWWIN v.1.68 in EPISUITE gives an estimated log K_{ow} of 2.60. Both values were below the cut-off value of 4 for bioaccumulation.

Aquatic toxicity

The only data on the aquatic toxicity on MeS is from an OECD TG 201 algae test giving a 72 h E_rC_{50} of 1.6 mg/L and a 72 h NOEC of 0.79 mg/L as geometric mean measured concentrations. No EC_{10} value is available. The assessment report on the original test report also gave results using measured concentrations at 49 h: 72 h E_rC_{50} of 1.475, 72 h E_rC_{10} of 1.033 mg/L and 72 h NOEC of 0.79 mg/L.

RAC agrees with the DS to read-across aquatic toxicity data from ethyl salicylate. RAC also supports the DS's view that data on SA shows that the 2–hydroxybenzoic acid is less toxic than the methyl ester, and therefore that the toxicity of the substituted 2-hydroxybenzoic form is proportional to the length of the substituent. MeS was expected to be less toxic than ethyl salicylate which is supported by the ECOSAR v.1.11 estimations. For ethyl salicylate, there is information for fish and *D. magna*. For fish, there is an OECD TG 203 fish test available giving a 96 h LC_{50} of 19.8 mg/L. The DS considered the test valid but the test report does not give information on nominal concentrations to allow the comparison with the measured ones. The DS observed that in every replicate considered separately the concentration of test substance was satisfactorily maintained during the test. For *D. magna* there is an OECD TG 202 test giving a

48 h EC_{50} of 28 mg/L based on initial measured concentrations. The concentrations have not been followed during the test.

Assessing the data on methyl and ethyl salicylate together there were reliable acute aquatic toxicity data on algae and fish and reliable chronic aquatic toxicity data on algae.

The DS based their classification proposal on the chronic algae data namely a 72 h NOEC of 0.79 mg/L. RAC agrees with this approach.

RAC has gathered data on methyl and ethyl salicylate via ECOSAR v.1.11 estimations to complement the very scarce database on MeS aquatic toxicity. The data is presented in the BD. The estimations show a great variability depending on the class of the substance and the endpoint in question.

Comparison with CLP criteria

Acute aquatic hazards

RAC agrees with the DS that **no acute aquatic classification is warranted** for MeS. RAC's opinion is based on the algae study result 72 h E_rC_{50} of 1.6 mg/L.

Chronic aquatic hazards

RAC agrees with the DS that MeS warrants classification as **Aquatic Chronic 3; H412** based on the 72 h NOEC of 0.79 mg/L and rapid degradability of the substance. The ECOSAR v.1.11 calculations show that algae are not necessarily the most sensitive trophic level. Thus, the classification might have to be revisited in case of new information.

ANNEXES:

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

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