Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

methyl 2,5-dichlorobenzoate

EC number: 220-815-7
CAS number: 2905-69-3

CLH-O-0000003156-78-01/A1

Adopted

28 November 2012

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Methyl 2,5-dichlorobenzoate***
EC number:	***220-815-7***
CAS number:	***2905-69-3***
Annex VI Index number:	**-**
Degree of purity:	***> 99.5 %***
Impurities:	***no relevant impurities***

1.2 Harmonised classification and labelling proposal

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

	CLP	**DSD**
Current entry in Annex VI of CLP Regulation (EC) No 1272/2008	-	-
Original proposal for consideration by RAC	Acute Tox. 4; H302 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 M-factor = 1	Xn; R22 N, R50-53 Concentration Classification C ≥ 25% N; R50-53 2.5% ≤ C < 25% N; R51-53 0.25% ≤ C < 2.5% R52-53 where C is the concentration of Methyl 2,5 dichlorobenzoate in the preparation
Amended proposal for consideration by RAC following Public Consultation	Acute Tox. 4; H302 Aquatic Chronic 2; H411	Xn; R22 N, R51-53 Concentration Classification C ≥ 25% N; R51-53 2.5% ≤ C < 25% R52-53 where C is the concentration of Methyl 2,5 dichlorobenzoate in the preparation
Resulting harmonised classification (future entry in Annex VI of CLP Regulation) as proposed by dossier submitter	Acute Tox. 4; H302 Aquatic Chronic 2; H411	Xn; R22 N, R51-53 Concentration Classification C ≥ 25% N; R51-53 2.5% ≤ C < 25% R52-53 where C is the concentration of Methyl 2,5 dichlorobenzoate in the preparation

The criteria of the 2nd ATP to the CLP Regulation have been considered.

As there is no valid long-term ecotoxicological data available, the 2nd ATP does not change the proposed classification for environmental hazards.

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				
2.2.	Flammable gases				
2.3.	Flammable aerosols				
2.4.	Oxidising gases				
2.5.	Gases under pressure				
2.6.	Flammable liquids				
2.7.	Flammable solids				
2.8.	Self-reactive substances and mixtures				
2.9.	Pyrophoric liquids				
2.10.	Pyrophoric solids				
2.11.	Self-heating substances and mixtures				
2.12.	Substances and mixtures which in contact with water emit flammable gases				
2.13.	Oxidising liquids				
2.14.	Oxidising solids				
2.15.	Organic peroxides				
2.16.	Substance and mixtures corrosive to metals				
3.1.	Acute toxicity - oral	Acute Tox. 4; H302			
	Acute toxicity - dermal				Conclusive but not sufficient for classification
	Acute toxicity - inhalation				Data lacking
3.2.	Skin corrosion / irritation				Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation				Inconclusive
3.4.	Respiratory sensitisation				Data lacking

3.4.	Skin sensitisation				Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity				Conclusive but not sufficient for classification
3.6.	Carcinogenicity				Data lacking
3.7.	Reproductive toxicity				Data lacking
3.8.	Specific target organ toxicity –single exposure				Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure				Conclusive but not sufficient for classification
3.10.	Aspiration hazard				Data lacking
4.1.	Hazardous to the aquatic environment	Aquatic Chronic 2; H411			
5.1.	Hazardous to the ozone layer				Data lacking

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

Labelling:

Pictograms:	GHS07, GHS09	
Signal Word:	Warning	
Hazard statements:	H302	Harmful if swallowed
	H411	Toxic to aquatic life with long lasting effects
Precautionary statements:	(P102)	Keep out of reach of children
	P264	Wash … thoroughly after handling
	P273	Avoid release to the environment
	P301 + P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
	P330	Rinse mouth
	P391	Collect spillage
	P501	Dispose of contents/container to …

Proposed notes assigned to an entry:

None

Table 4: Proposed classification according to DSD

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness				
Oxidising properties				
Flammability				
Other physico-chemical properties *[Add rows when relevant]*				
Thermal stability				
Acute toxicity	Xn; R22			
Acute toxicity – irreversible damage after single exposure				Conclusive but not sufficient for classification
Repeated dose toxicity				Conclusive but not sufficient for classification
Irritation / Corrosion				Inconclusive
Sensitisation				Conclusive but not sufficient for classification
Carcinogenicity				Data lacking
Mutagenicity – Genetic toxicity				Conclusive but not sufficient for classification
Toxicity to reproduction – fertility				Data lacking
Toxicity to reproduction – development				Data lacking
Toxicity to reproduction – breastfed babies. Effects on or via lactation				Data lacking
Environment	N; R51-53	25 % ≤ Cn [3)] classification of preparation is N; R51-53 2.5 % ≤ Cn < 25 % classification of preparation is R52-53		

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification
[3)] Cn is the concentration of methyl 2,5-dichlorobenzoate in the preparation

Labelling: Hazard Symbols,
Indications of danger: Xn Harmful
N Dangerous for the environment
R-phrases: R22 Harmful if swallowed
R51/53 Toxic to aquatic organisms, may cause long-term adverse effects to the aquatic environment
S-phrases: (S2) Keep out of the reach of children
S22 Do not breathe dust
S60 This material and its container must be disposed of as hazardous waste
S61 Avoid release to the environment. Refer to special instructions/ safety data sheets

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

2.2 Short summary of the scientific justification for the CLH proposal

2.3 Current harmonised classification and labelling

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

There is no entry for methyl 2,5-dichlorobenzoate available in Annex VI, Table 3.1 in the CLP Regulation.

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

There is no entry for methyl 2,5-dichlorobenzoate available in Annex VI, Table 3.2 in the CLP Regulation.

2.4 Current self-classification and labelling

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

2.4.2 Current self-classification and labelling based on DSD criteria

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Methyl 2,5-dichlorobenzoate is an active substance in the meaning of Directive 91/414/EEC and therefore subject to harmonised classification and labelling (Regulation EC 1272/2008 article 36.2).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

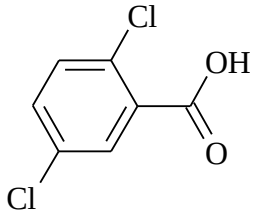
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

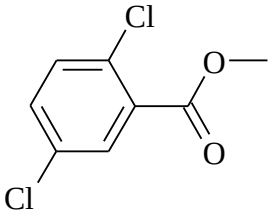
Table 5: Substance identity

EC number:	220-815-7
EC name:	methyl 2,5-dichlorobenzoate
CAS number (EC inventory):	2905-69-3
CAS number:	2905-69-3
CAS name:	Benzoic acid, 2,5-dichloro-, methyl ester
IUPAC name:	methyl 2,5-dichlorobenzoate
CLP Annex VI Index number:	-
Molecular formula:	$C_8H_6Cl_2O_2$
Molecular weight range:	205

Structural formula:



Säure



Methylester

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
methyl 2,5-dichlorobenzoate	> 99.5		

1.3 Physico-chemical properties

Table 7: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	yellow crystals	Draft Assessment Report	
Melting/freezing point	34.6 °C		
Boiling point	250.6 °C		
Relative density	1.48		
Vapour pressure	370 Pa at 25 °C		
Surface tension	ca. 60 mN/m at concentration 8 mg/L		measured at 10 % of saturation concentration
Water solubility	0.087 g/L at 20 °C		
Partition coefficient n-octanol/water	3.46 at 20 °C		
Flash point	133 °C		
Flammability	not flammable		
Explosive properties	no explosive properties		
Self-ignition temperature	no up to the melting point		
Oxidising properties	no oxidising properties		
Granulometry	not available	-	
Stability in organic solvents and identity of relevant degradation products	not available	-	
Dissociation constant	not relevant	-	
Viscosity	not available	-	

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

Plant growth regulator and fungicide for grafting of grapevines.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 8: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference

Note: Table left open by dossier submitter

3.1 Physico Chemical Properties

3.1.1 Summary and discussion of Physico chemical properties

3.1.1.1 Dossier submitter

Due to the physico-chemical properties of methyl 2,5-dichlorobenzoate a classification is not necessary in this area (data conclusive, but not sufficient for classification).

3.1.1.2 RAC evaluation

Although originally not addressed, following a comment during public consultation the dossier submitter stated that the available data indicate that a classification for physico-chemical properties is not necessary. RAC supported the non-classification for physico-chemical properties, as methyl 2,5-dichlorobenzoate is not explosive, not flammable, has no self-ignition up to the melting point and has no oxidising properties (see Table 7).

4 HUMAN HEALTH HAZARD ASSESSMENT

In this report, only summaries are given. A more extensive description of the studies and of the observed findings are included in the draft assessment report, which is attached to the IUCLID dossier.

There are no toxicological studies performed with impurities. The technical active substance methyl 2,5-dichlorobenzoate used in formulations is equivalent to methyl 2,5-dichlorobenzoate that has been used in the toxicological studies. The chemical composition of both is similar. Any component other than the pure active substance, which is present in the technical active substance as manufactured (impurities including non-active isomers) originating from the manufacturing process or from degradation during storage is covered by the toxicological studies. Therefore, no further toxicological studies with impurities have been performed.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Orally administered methyl 2,5-dichlorobenzoate (2,5-dichlorobenzoic acid methylester = 2,5-DCBME) was almost completely absorbed in rats. 2,5-DCBME was rapidly metabolised and eliminated. Regardless of the route of administration and the dose, roughly the complete radioactivity was eliminated within 24 h. Faecal excretion is negligible and plays only a minor role at the high dose. Besides the excretion relevant organs, no particular accumulation in other organs has been observed. No sex specific differences in the elimination occurred. The free acid and the glycine conjugate were identified and characterised as major metabolites. In addition, acylglucuronide-isomers were detected dose dependently. In the 24 h urine samples the recovered radioactivity of the total dose ranged 73.4 % for 2,5-dichlorobenzoic acid (M11.7) followed by 2,5-dichlorobenzoylglycine (M7.2) with 18.6 % and the three acylglucuronide-isomers amounted each up to 2 % following 1ow dose administration (Ferser-Zügner, 2004 ASB2007-1336).

4.1.2 Human information

No other relevant information is available.

4.1.3 Summary and discussion on toxicokinetics

In rat the absolute bioavailability of 2,5-DCBME was almost 100 % comparing the total renal excretion of oral and intravenous application indicating a complete absorption. 2,5-DCBME was rapidly metabolised and eliminated within 24 h. Faecal excretion is negligible and plays only a minor role at the high dose. Besides the excretion relevant organs no particular accumulation in other organs has been observed. No sex specific differences in the elimination occurred. As major metabolites the free acid and the glycine conjugate were identified and characterised. In addition, acylglucuronide-isomers were detected dose dependently.

4.2 Acute toxicity

4.2.1 Non-human information

The results of the acute toxicity studies are summarised in Table 9.

Table 9: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Acute oral LD_{50}, Rat / Wistar	LD_{50}: 1175 mg/kg bw males LD_{50}: 1030 mg/kg bw females Mortality: ≥ 750 mg/kg bw after 24 hours		(Dickhaus et al., 1982 TOX2002-544)
Acute oral LD_{50}, Mouse / CFI	LD_{50}: 910 mg/kg bw males & females Mortality: ≥ 700 mg/kg bw after 24 hours		(Dickhaus et al., 1982 TOX2002-545)
Acute dermal LD_{50}, Rat / Wistar	LD_{50}: > 10000 mg/kg bw		(Dickhaus et al., 1982 TOX2002-546)
Acute inhalation LC_{50}, Rat / CRL:(WI) BR	Not determined	No spray or dust feasibly	(Hirka et al., 2004 ASB2007-1347)

4.2.1.1 Acute toxicity: oral

The acute oral toxicity of 2,5-DCBME was in same order of magnitude in rats and mice. The acute oral LD50 was 1030 mg/kg bw in rats and 910 mg/kg bw in mice. In rats in all dosage groups essentially abdominal ache syndrome, exophthalmus, gasping, ataxia, disturbances of coordination were observed already a few minutes post application. These symptoms changed into sedation and coma. Mortality was observed at ≥ 750 mg/kg bw in rats. In mice in all dosage groups essentially abdominal ache syndrome, reduced activity, reduced frequency of respiration, titubation, tremor, piloerection, convulsions and reduced readiness for reflexing was observed. During the following time of observation the surviving mice showed sedation and reduced readiness for reflexing. Mortality was observed at ≥ 700 mg/kg bw in mice.

4.2.1.2 Acute toxicity: inhalation

Due to technical problems to reach the necessary concentrations in the aerosol caused by the physico-chemical properties of the active substance (e.g. low melting point, poor solubility in water) it was not feasible to perform spray or dust of satisfying concentration. Also trials with melted material failed. It was impossible to produce continuous mass flow for duration of more than 10 minutes although different nebulisers of several producers were tested. The study was interrupted due to technical reasons.

4.2.1.3 Acute toxicity: dermal

The dermal toxicity in rats was low. The acute dermal LD50 in rats was greater than 10000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

No other relevant information is available.

4.2.2 Human information

No other relevant information is available.

4.2.3 Summary and discussion of acute toxicity

4.2.3.1 Dossier submitter

The acute oral toxicity of 2,5-DCBME was in the same order of magnitude in rats and mice. The acute oral LD_{50} was 1030 mg/kg bw in rats and 910 mg/kg bw in mice. Mortality was observed at ≥ 750 mg/kg bw in rats and at ≥ 700 mg/kg bw in mice. The acute dermal LD_{50} in rats was greater than 10.000 mg/kg bw. The acute inhalation toxicity in rats could not be determined because use of spray or dust was not feasible in the test.

Comparison with criteria

Table 10 presents the toxicological results in comparison with DSD and CLP criteria.

Table 10: Comparison of the toxicological results

Toxicological result	CLP criteria	DSD criteria
Oral LD_{50}, rat, males: 1175 mg/kg Oral LD_{50}, rat, females: 1030 mg/kg $LD_{50,}$ mouse: 910 mg/kg bw, males & females	Cat. 4: 300 < LD_{50} ≤ 2000 mg/kg (oral)	Harmful: LD_{50} per oral, rat: 200 < LD_{50} ≤ 2000 mg/kg
Inhalation LC_{50}, rat: Not determined (no spray or dust feasible)	-	-
Dermal $LD_{50,}$ rat: > 10.000 mg/kg	Cat. 4: 1000 < LD_{50} ≤ 2000 mg/kg (dermal)	Harmful: LD_{50} dermal, rat or rabbit: 400 < LD_{50} ≤ 2000 mg/kg

Dossier submitter's conclusions on classification and labelling

The acute oral toxicity of 2,5-DCBME meets the CLP and DSD criteria. Based on the results of the acute oral toxicity studies 2,5-DCBME should be classified as Acute Tox. 4 (H302) according to CLP and as harmful, Xn; R22 "Harmful if swallowed" according to DSD.

The results of the acute dermal toxicity studies do not meet the CLP or DSD criteria. Classification and labelling of 2,5-DCBME concerning acute dermal toxicity is not required.

There are no results of the acute inhalation toxicity study to compare with the CLP and DSD criteria. No conclusion can be drawn on classification of 2,5-DCBME for acute inhalation toxicity.

4.2.3.2 RAC evaluation

During public consultation, support was expressed for the proposal.

Based on a comparison of the available LD_{50} values in rats and mice with the criteria, RAC supports the conclusion of the dossier submitter that 2,5-DCBME should be classified for acute oral toxicity (with **Acute Tox. 4 – H302** (CLP) and **Xn; R22** (DSD)), but not for acute dermal toxicity. In the absence of data, RAC agrees that no conclusion can be drawn on the classification of 2,5-DCBME for acute inhalation toxicity.

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

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

4.3.1.1 Dossier submitter

There are no relevant data relating to the classification of 2,5-DCBME for specific target organ toxicity by single exposure.

Dossier submitter's comparison with criteria

There are no relevant data to compare with criteria.

Dossier submitter's conclusions on classification and labelling
Classification and labelling is not required.

4.3.1.2 RAC evaluation

The evaluation by RAC relates to the proposal of the dossier submitter not to classify 2,5-DCBME for specific target organ toxicity – single exposure. During public consultation, one member state suggested that the findings of sedation and coma in the acute oral toxicity studies should be discussed in the context of the criteria for STOT SE.

In response to this comment, the dossier submitter argued that since the CLP guidance (3.8.2.1.2) states that human data or inhalation studies should be considered for STOT SE 3 (narcotic effects), no classification is proposed because effects were observed in *oral* studies.

RAC notes that this interpretation of the guidance is not correct, as the specific section states *"Although classification in **Category 3** is primarily based on human data, if available, animal data can be included in the evaluation. These animal data on RTI (respiratory tract irritation) and NE (narcotic effects) will generally come from standard acute inhalation studies, although it is possible that narcosis could be observed in studies using other routes."*

The guidance indicates (3.8.2.2.2) that *'narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia'*. In the oral single dose studies, all these symptoms were observed in rats and/or mice, as was sedation and (in rats) coma, at dose levels also resulting in mortality. Severe, but transient, ataxia was also observed in a 2-week oral dose-range-finding study in rats following dosing with 900 mg/kg bw/d (starting 10 minutes after dosing and lasting for 4-6 hours), a dose level that is close to the oral LD_{50} value of approximately 1000 mg/kg bw. No such effect was observed in that study at the next lower dose of 300 mg/kg bw/d, or in a rat oral 28-day study at doses of 100, 300 and 900 mg/kg bw/d. The latter study, however, showed reduced mobility in the form of paralysis of the hind legs at 300 and 900 mg/kg bw/d (as well as increased incidence of impaired gait and wire manoeuver, decreased sensitivity to toe pinch and tail pinch, decreased hind leg splay and limb rotation, decreased spontaneous locomotion movements, and decreased grip strengths of the fore and hind limbs, following a neurological assessment in week 4). The paralysis of the hind legs was seen from day 1 of treatment at 900 mg/kg bw/d, and from day 4 at 300 mg/kg bw/d. At both doses the effect was transient, lasting from 10 minutes to 6 hours after each dosing, and was no longer seen immediately after treatment was stopped on day 29.

From the available data it is clear that 2,5-DCBME is a neurotoxic substance, causing comparable effects in acute and repeated dose toxicity studies at doses that are within half an order of magnitude of each other. Looking at the onset and duration of the neurotoxic effects in the repeated dose studies, they do not seem more persistent than after acute exposure. Therefore, given their clearly transient nature, RAC considers the neurotoxic effects in the

repeated dose studies to be indicative of acute toxicity (see also section 1.6.2 for further explanation).

The observed narcotic/neurotoxic effects in the oral acute and repeated dose toxicity studies fulfil the criteria for STOT SE 3. Whereas RAC notes that some of the effects occur at or near lethal dose levels, and for lethality the substance is already proposed to be classified, RAC does not consider additional classification for STOT SE to be a "double classification", given that some other effects (paralysis in particular) occur below lethal dose levels. RAC therefore proposes to classify 2,5-DCBME for specific target organ toxicity – single exposure (with **STOT SE 3 – H336**), in order to flag its narcotic/neurotoxic properties. No classification under DSD is warranted, as under DSD the corresponding R-phrase R67 is for vapours/inhalation route only.

4.4 Corrosion / Irritation

4.4.1 Skin irritation

The results of the skin irritation study are summarised in Table 11.

Table 11: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation, Rabbit / White New Zealand	Slight reversible edema on the shaved and shaved/scarified skin in 5 out of 8 sacrificed animals	-	(Dickhaus et al., 1982 TOX2002-547)

4.4.1.1 Non-human information

After 24 hours a very slight reversible edema was observed on the shaved and shaved/scarified skin in 5 out of 8 sacrificed animals. According to the index of primary irritation of 0.31 the substance is judged to be mild irritant.

4.4.1.2 Human information

No relevant information is available.

4.4.1.3 Summary and discussion of skin irritation

4.4.1.3.1 Dossier submitter

Slight to moderate but transient signs of dermal irritation were noted after application to the skin of rabbits.

Comparison with criteria

Table 12 in comparison with DSD and CLP criteria.

Table 12: Summary of the toxicological results

Toxicological result	DSD criteria	CLP criteria
After 24 hours a erythema score of 1 was observed in 5/8 animals on the shaved skin. At the reading 72 h and 7 d post application, all scores were 0. Edema scores were 0 at all reading times.	R38 Irritating to skin: Significant inflammation of the skin which persists for at least 24 hours after an exposure period of up to four hours; mean value of the scores for either erythema and eschar formation or oedema formation, calculated over all the animals tested, is 2 or more	Category 2 Irritant: Mean value of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Conclusions on classification and labelling

Slight to moderate but transient signs of dermal irritation were noted after application to the skin of rabbits. After 24 hours an erythema score of 1 was observed in 5/8 animals on the shaved skin. At 72 h and 7 d post application, all scores were 0. Oedema scores were 0 at all reading times. Since the mean values of the readings after 24 to 72 hours after application were below the thresholds defined in CLP and DSD, classification of 2,5-DCBME for skin irritation is not required.

4.4.1.3.2 RAC evaluation

The evaluation by RAC relates to the proposal of the dossier submitter not to classify 2,5-DCBME for skin irritation. This proposal was not specifically commented on during public consultation.

In a skin irritation study with rabbits only slight, transient irritation was observed. For erythema, a maximum score of 1 was found in 5/8 animals after 24 h, both for intact and for abraded skin. At 72 h and 7 d post application, the scores for erythema were all 0. Oedema scores were 0 at all reading times. The mean score for erythema was below the threshold value of 2.3 for Skin Irrit. 2 – H315 (CLP) or 2 for Xi; R38 (DSD). RAC therefore supports the conclusion of the dossier submitter that 2,5-DCBME should not be classified for skin irritation.

4.4.2 Corrosivity

There is no evidence of corrosivity of 2,5-DCBME (see 4.4).

4.4.2.1 Non-human information

No relevant data.

4.4.2.2 Human information

No relevant data.

4.4.2.3 Summary and discussion of corrosivity

Dossier submitter

There are no relevant data to discuss corrosivity of 2,5-DCBME.

Comparison with criteria

There are no relevant data to compare with criteria.

Conclusions on classification and labelling

Classification and labelling is not required.

RAC evaluation

The evaluation by RAC relates to the proposal of the dossier submitter not to classify 2,5-DCBME for corrosive properties. This proposal/endpoint was not specifically commented on during public consultation.

In the skin and eye irritation studies, no indications were found for a corrosive effect of 2,5-DCBME. RAC therefore supported the conclusion of the dossier submitter that 2,5-DCBME should not be classified for corrosivity.

4.4.3 Eye corrosion/irritation

The results of the eye irritation study are summarised in Table 13.

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation, Rabbit / White New Zealand	Up to 8 hours after the application the conjunctiva showed redness, chemosis and secretion; after 24 hours post application no irritations	10 % dilution of the substance	(Dickhaus et al., 1982 TOX2002-548)

4.4.3.1 Non-human information

The substance 2,5-DCBME was tested diluted in a primary eye irritation test in rabbit eye. 2,5-DCBME was diluted 10 %. Up to 8 hours after the application the conjunctiva showed redness, chemosis and secretion. After 24 hours post application no irritations have been observed.

Individual scores in animals treated with the 10 % solution of 2,5-DCBME (Dickhaus et al., 1982 TOX2002-548):

		Hour / Stunden					Day / Tage					
		1	2	4	8	24	2	3	4	5	6	7
Tier-Nr. 1												
1. Cornea	A	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0
2. Iris	A	0	0	0	0	0	0	0	0	0	0	0
3. Conjunktiva	A	2	1	1	1	0	0	0	0	0	0	0
	B	2	2	1	0	0	0	0	0	0	0	0
	C	3	2	2	0	0	0	0	0	0	0	0
Tier-Nr. 2												
1. Cornea	A	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0
2. Iris	A	0	0	0	0	0	0	0	0	0	0	0
3. Conjunktiva	A	1	1	1	1	0	0	0	0	0	0	0
	B	2	2	1	0	0	0	0	0	0	0	0
	C	2	2	2	1	0	0	0	0	0	0	0
Tier-Nr. 3												
1. Cornea	A	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0
2. Iris	A	0	0	0	0	0	0	0	0	0	0	0
3. Conjunktiva	A	1	1	1	1	0	0	0	0	0	0	0
	B	1	1	1	0	0	0	0	0	0	0	0
	C	3	3	2	1	0	0	0	0	0	0	0

		Stunden					Tage					
		1	2	4	8	24	2	3	4	5	6	7
Tier-Nr. 4												
1. Cornea	A	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0
2. Iris	A	0	0	0	0	0	0	0	0	0	0	0
3. Conjunktiva	A	2	1	1	1	0	0	0	0	0	0	0
	B	2	2	1	1	0	0	0	0	0	0	0
	C	2	2	2	1	0	0	0	0	0	0	0
Tier-Nr. 5												
1. Cornea	A	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0
2. Iris	A	0	0	0	0	0	0	0	0	0	0	0
3. Conjunktiva	A	1	1	1	1	0	0	0	0	0	0	0
	B	2	2	1	0	0	0	0	0	0	0	0
	C	2	2	1	1	0	0	0	0	0	0	0
Tier-Nr. 6												
1. Cornea	A	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0
2. Iris	A	0	0	0	0	0	0	0	0	0	0	0
3. Conjunktiva	A	2	1	1	1	0	0	0	0	0	0	0
	B	2	2	1	0	0	0	0	0	0	0	0
	C	3	2	2	1	0	0	0	0	0	0	0

		Stunden					Tage					
		1	2	4	8	24	2	3	4	5	6	7
Tier-Nr. 7												
1. Cornea	A	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0
2. Iris	A	0	0	0	0	0	0	0	0	0	0	0
3. Conjunktiva	A	2	1	1	1	0	0	0	0	0	0	0
	B	2	2	1	1	0	0	0	0	0	0	0
	C	2	2	1	0	0	0	0	0	0	0	0
Tier-Nr. 8												
1. Cornea	A	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0
2. Iris	A	0	0	0	0	0	0	0	0	0	0	0
3. Conjunktiva	A	1	1	1	1	0	0	0	0	0	0	0
	B	1	1	1	0	0	0	0	0	0	0	0
	C	2	1	1	0	0	0	0	0	0	0	0

4.4.3.2 Human information

No other relevant information is available.

4.4.3.3 Summary and discussion of eye corrosion/irritation

Dossier submitter

Slight to moderate but transient signs of ocular irritation were noted after application to the eyes of rabbits. In the eye irritation study, only a 10 % dilution of the substance was used. The authors of the study as well as the PRAPeR Expert Meeting (PRAPeR Expert Meeting 54 Sub-group 2 (07 – 11 July 2008) 11 July 2008, Dichlorobenzoic acid) proposed to classify the product containing 10 % of the test substance as "slightly irritant" as a precaution, because it could not be ruled out that the concentrate would not lead to stronger irritation to the eyes (EFSA Scientific Report (2008) 180, 1-50, Conclusion on the peer review of 2,5-dichlorobenzoic acid methylester).

Dossier submitter's comparison with criteria

Up to 8 hours after the application, the conjunctiva showed redness, chemosis and secretion; 24 hours post application there was no evidence of irritation. The mean values of the readings after 24 to 72 hours after application were below the thresholds defined in CLP for Eye irritation, category 2 (positive response of corneal opacity ≥ 1 and/or iritis ≥ 1, and/or conjunctival redness ≥ 2 and/or conjunctival oedema (chemosis) ≥ 2; in at least in 2 of 3 tested animals, calculated as the mean scores following grading at 24, 48 and 72 h, and fully reversible within an observation period of 21 days); or Xi; R36, Irritating to eyes according to DSD (Significant ocular lesions within 72 h and persisting for at least 24 h, corneal opacity ≥ 2 but < 3, iris lesion ≥ 1 but < 1,5, redness of the conjunctivae ≥ 2,5, oedema of the conjunctivae (chemosis) ≥ 2).

Dossier submitter's conclusions on classification and labelling

Slight to moderate but transient signs of ocular irritation were noted after application of a 10 % dilution of 2,5-DCBME to the eyes of rabbits. The mean values of the readings after 24 to 72 hours after application were below the thresholds defined in CLP and DSD However, no conclusion can be drawn on the classification of 2,5-DCBME because only a 10 % dilution of 2,5-DCBME was tested.

RAC evaluation

The evaluation by RAC relates to the proposal of the dossier submitter not to classify 2,5-DCBME for eye irritation, in absence of data on 2,5-DCBME in a more concentrated form than 10%. During public consultation, support was expressed for the proposal.

In the eye irritation study with rabbits, a 10% solution of 2,5-DCBME caused slight to moderate and transient signs of ocular irritation. Conjunctival redness, chemosis and secretion (scores 1-3) was seen up to 8 h after application in all 8 animals tested, but scores for these effects were all 0 from 24 h up to 7 d post application. Methyl 2,5-dichlorobenzoate did not produce effects on the cornea or iris (scores 0 at all reading times). Based on these results, classification for eye irritation for a 10% solution of 2,5-DCBME is not warranted. This conclusion was also drawn by EFSA in their peer review of 2,5-DCBME in 2008. Yet, as a precaution EFSA proposed to classify 2,5-DCBME for eye irritation with Xi; R36, because it could not be ruled out that a more concentrated form would not lead to a stronger irritation to the eyes. Rather than proposing a precautionary classification, RAC concludes that in the absence of appropriate data, no conclusion can be drawn on the classification for eye irritation.

4.4.4 Respiratory tract irritation

4.4.4.1 Non-human information

There are no relevant data.

4.4.4.2 Human information

There are no relevant data.

4.4.4.3 Summary and discussion of respiratory tract irritation

Dossier submitter

There are no data relevant to the respiratory tract irritation classification.

Dossier submitter's comparison with criteria
There are no relevant data to compare with criteria.

Dossier submitter's conclusion on classification and labelling
No conclusion can be drawn on respiratory tract irritation.

RAC evaluation

The evaluation by RAC relates to the proposal of the dossier submitter not to classify 2,5-DCBME for respiratory tract irritation, due to a lack of data. This proposal/endpoint was not specifically commented on during public consultation.

In the absence of data, RAC agrees with the dossier submitter that no conclusion can be drawn on the classification for respiratory tract irritation.

4.5 Sensitisation

4.5.1 Skin sensitisation

4.5.1.1 Non-human information

The results of the skin sensitisation study are summarised in Table 14.

Table 14: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Skin Sensitisation (Maximisation Test), Guinea pigs / Dunkin Hartley	Not sensitising	-	(Stahl, 2005 ASB2007-1376)

According to the observations of this study no symptoms of skin sensitisation could be observed. The mean rate of scores was 0 % after 24 h and 48 h.

4.5.1.2 Human information

No relevant data are available.

4.5.1.3 Summary and discussion of skin sensitisation

Dossier submitter

In a maximisation test by Magnusson and Kligman no symptoms of skin sensitisation could be observed.

Comparison with criteria

There are no relevant data to compare with criteria.

Conclusions on classification and labelling

Classification and labelling is not required.

RAC evaluation

The evaluation by RAC relates to the proposal of the dossier submitter not to classify 2,5-DCBME for skin sensitisation. During public consultation, this proposal/endpoint was not specifically commented on.

2,5-DCBME (0.1% at intradermal induction, 75% at topical application after treatment of skin with 10% sodium lauryl sulfate) tested negative in a guinea pig maximisation test according to Magnusson and Kligman. None of the animals (test and control) showed any skin reaction. RAC therefore supports the conclusion of the dossier submitter that 2,5-DCBME should not be classified for skin sensitisation.

4.5.2 Respiratory sensitisation

4.5.2.1 Non-human information

No relevant data are available.

4.5.2.2 Human information

No relevant data are available.

4.5.3 Summary and discussion of respiratory sensitisation

Dossier submitter

There are no relevant data to discuss respiratory sensitisation.

Comparison with criteria

There are no relevant data to compare with criteria.

Conclusions on classification and labelling

No conclusion can be drawn on respiratory sensitisation potential.

RAC evaluation

The evaluation by RAC relates to the proposal of the dossier submitter not to classify 2,5-DCBME for respiratory sensitisation due to absence of data. During public consultation, this proposal/endpoint was not commented on.

In the absence of data, RAC agrees that no conclusion can be drawn on the classification for respiratory sensitisation.

4.6 Repeated dose toxicity

Table 15: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
2-week dose-range-finding study in rats, 100, 300 or 900 mg/kg bw/day by gavage	900 mg/kg bw/day: ataxia, decreased body weight and food consumption NOAEL: 300 mg/kg bw/day	-	(Leuschner, 2004 ASB2007-1381)
28 days subacute toxicity in rats, 100, 300 or 900 mg/kg bw/day by gavage	≥ 300 mg/kg bw/day: reduced motility, impaired gait and wire maneuver, decreased sensitivity to toe pinch and tail pinch, decreased hind leg splay and limb rotation, decreased spontaneous locomotion and grip strength of the fore- and hindlimbs, increased liver weight 900 mg/kg bw/day: pilo-erection, in males reduced body weight, reduced haemoglobin content, number of erythrocytes and haematocrit values, reduced plasma levels of glucose and potassium, increased activity of alanine aminotransferase, in females reduced cholesterol plasma, increased kidney weight, fatty infiltrations in the heart, in males increased oligospermia in the epididymis. NOAEL: 100 mg/kg bw/day	-	(Leuschner, 2004 ASB2007-1382)

4.6.1 Non-human information

4.6.1.1 Repeated dose toxicity: oral

The toxicity of 2,5-DCBME was investigated in a 2-week study to assist the selection of dose levels for a 28-day study in rats. Administration of 900 mg 2,5-DCBME/kg bw/day caused severe ataxia in all animals starting 10 minutes after administration. The symptoms lasted for 4-6 hours. Also at the high dose group the body weight was decreased by 14 % and 20 % (test weeks 1 and 2, respectively) for the male animals (statistically significant at $p \leq 0.01$) and by 7 % for the female animals (statistically not significant). The food consumption was 11 % to 18 % (males) and up to 13 % (females) below the control group values. No effects were

observed in dose groups 100 and 300 mg/kg bw/day. The macroscopic post mortem examination on test day 15 did not reveal any test item-related findings. The NOAEL of the study is 300 mg/kg bw/day. On basis of the results dose levels were selected for a 28-day oral study in rats (Leuschner, 2004 ASB2007-1381).

In the 28-day study the test compound was administered once daily by gavage (7 day per week) at doses of 100, 300 and 900 mg/kg bw/day. None of the rats died prematurely.

Animals treated with 300 or 900 mg/kg bw/d showed reduced motility in form of a paralysis of the hind legs from test day 4 or test day 1 onwards to test day 28, respectively, lasting 10 minutes to 6 hours after application. In addition, pilo-erection was noted in all 10 male and 10 female animals of the high dose group during the first treatment week, 4 of 10 males and 2 of 10 females showed an abdominal position from test day 1 to 4. Neurological screening was done. Animals treated with 300 or 900 mg/kg bw/d showed a dose-related increased incidence of impaired gait and wire maneuver, a decreased sensitivity to toe pinch and tail pinch, and a decreased hind leg splay and limb rotation. In addition, a dose-related significant decrease was noted for the slight and active movements of the spontaneous locomotion and in the grip strength of the fore- and hindlimbs. Male animals treated with 900 mg/kg bw/day showed a reduced body weight a reduced haemoglobin content, a reduced number of erythrocytes, a reduced haematocrit value in test week 4, reduced plasma levels of glucose and potassium and an increased activity of alanine aminotransferase. Female animals of the high dose group showed a reduced cholesterol plasma level at the end of the treatment period. At 300 mg/kg bw/day, an increase in the absolute and relative liver weight was noted for the males. Animals treated with 900 mg/kg bw/day showed an increase in the relative organ weights of the liver for male and female animals, the absolute liver weight of females and of relative kidney weight of the female animals. Animals treated with 900 mg/kg bw/day revealed fatty infiltrations in the heart. Male animals showed an increased oligospermia in the epididymis. Body weight of the male animals did not normalise during the 6-week recovery period. The body weight remained 17 % below the control group. Other findings noted at the end of the treatment period had completely subsided at the end of the 6-week recovery period. The no observed adverse effect level (NOAEL) of the 28-day study was 100 mg/kg bw/day (Leuschner, 2004 ASB2007-1382).

4.6.1.2 Repeated dose toxicity: inhalation

No data are available.

4.6.1.3 Repeated dose toxicity: dermal

No data are available.

4.6.1.4 Repeated dose toxicity: other routes

No data are available.

4.6.1.5 Human information

No data are available.

4.6.1.6 Other relevant information

No other relevant information is available.

4.6.1.7 Summary and discussion of repeated dose toxicity

Dossier submitter

The toxicity of 2,5-dichloro benzoic acid methylester was investigated in a 2-week dose-range finding study and a 28-day study, both in rats. In the 2-week study, administration of 900 mg 2,5-dichlorobenzoic acid methylester per kg bw/d caused severe ataxia in all animals starting 10 minutes after administration. The symptoms lasted for 4-6 h. The body weight was decreased relative to the controls. The food consumption was lower relative to the control group. The NOAEL of the study was 300 mg/kg bw/d.

In the 28-day study the test compound was administered once daily by gavage (7 d/week) at doses of 100, 300 and 900 mg/kg bw/d. Animals treated with 300 or 900 mg/kg bw/d showed reduced mobility in form of a paralysis of the hind legs. In addition, pilo-erection was noted in the high dose group. Animals treated with 300 or 900 mg/kg bw/d showed a dose-related increased incidence of impaired gait and wire manoeuvre, a decreased sensitivity to toe pinch and tail pinch, and a decreased hind leg splay and limb rotation. In addition, a dose-related significant decrease was noted for the slight and active movements of the spontaneous locomotion and in the grip strength of the fore and hind limbs. Male animals treated with 900 mg/kg bw/d showed a reduced body weight and effects on parameters of haematology and clinical chemistry. At 300 mg/kg bw/d, an increase in the relative liver weight was noted for the males. Animals treated with 900 mg/kg bw/d showed an increase in the organ weights of the liver of male and female animals and of kidneys of the female animals. Animals treated with 900 mg/kg bw/d revealed fatty infiltrations in the heart. Male animals showed an increased oligospermia in the epididymis. Body weight of the male animals did not normalise during the 6-week recovery period. The body weight remained 17 % below the control group. Other findings noted at the end of the treatment period had completely subsided at the end of the 6-week recovery period. The NOAEL of the 28-day study was 100 mg/kg bw/d.

There are no relevant findings in the 2-week and 28-day studies to discuss classification concerning specific target organ toxicity - repeated exposure (CLP) or repeated dose toxicity (DSD).

Dossier submitter's comparison with criteria

There are no relevant findings to compare with criteria for classification according to CLP or DSD.

Dossier submitter's conclusions on classification and labelling

There are no findings relevant for classification according to CLP or DSD.

RAC evaluation

During public consultation, one member state suggested that the findings of (seemingly transient) neurotoxic effects in the 28-day study should be discussed in the context of criteria and adjusted guidance values for STOT RE.

In response to this comment, the dossier submitter argued that the effects were fully reversible (i.e. signs of neurotoxicity occurred directly after gavage from day one onwards, and lasted from ten minutes to a few hours) and were not severe, and that therefore a classification for STOT RE is not proposed. The dossier submitter further argued that guidance values are not to be regarded as strict demarcation values.

In the 28-day toxicity study, neurotoxic effects were the most sensitive effects observed: they were observed at 300 and 900 mg/kg bw/d (reduced mobility in the form of paralysis of the hind legs and, following a neurological assessment in week 4, increased incidence of impaired gait and wire manoeuver, decreased sensitivity to toe pinch and tail pinch, decreased hind leg splay and limb rotation, decreased spontaneous locomotion movements, and decreased grip strengths of the fore- and hind limbs), whereas other effects were mainly noted at 900 mg/kg bw/d. The paralysis of the hind legs was transient, lasting 10 minutes to 6 hours after each

dosing, and was no longer seen immediately after treatment was stopped on day 29. At 900 mg/kg bw/d it took one dose to manifest, but at 300 mg/kg bw/d four doses were necessary. Neurotoxicity (severe, but transient ataxia) was also seen in the 2-week dose-range finding study, but only at the highest dose of 900 mg/kg bw/d.

Given that at 300 mg/kg bw/d the paralysis took four days to manifest, RAC considered whether this was an indication of repeated dose toxicity, or whether it was in fact more a sign of acute toxicity. The latter possibility was raised because of the clearly transient nature of the paralysis and the fact that disturbances of coordination and other neurotoxic effects such as ataxia were also observed in the acute toxicity studies at only slightly higher (2.5-fold) doses of 2,5-DCBME.

After this issue had been raised by RAC, as further explanation of the nature of the neurotoxic effects observed, Industry referred to a WHO evaluation of (a.o.) benzoates and benzoic acid (WHO, 1997). In this evaluation, the toxic effects induced upon exposure to high doses of these substances are linked to glycine depletion. Benzoates are metabolised to benzoic acid, which in turn is conjugated with glycine to hippuric acid (=benzoylglycine). This conjugation is a saturable process in which the availability of glycine is the rate limiting step. Therefore, high doses result in glycine depletion leading to toxic effects, including neurotoxicity. Supplementation with glycine was shown to alleviate the toxic effects.

It was argued that the above explanation is consistent with findings in the 28-day study for 2,5-DCBME, the metabolism and elimination of which is rapid (within 24 h), without accumulation in organs/tissues, and for which the major metabolites have been identified as the free acid (2,5-dichlorobenzoic acid) and the glycine conjugate (2,5-dichlorobenzoylglycine). The high dose group (900 mg/kg bw, which is close to the LD_{50}) in the 28-day study showed effects from day 1 whereas the lower dose group (300 mg/kg bw) showed effects only after 4 days (after depletion of the glycine pool). The effects lasted only 10 minutes to 6 hours after application and all animals showed a total recovery from day 29 on when no further test substance was administered. Therefore, Industry is of the opinion that the (clearly severe) effects should be regarded as acute toxicity on each single day because on each day after cessation of treatment, further recovery was observed. They consider a classification for STOT RE 2 not justified, as there are definitely no signs for repeated toxicity, given also the rapid metabolism and elimination and the absence of accumulation.

Based on an overall weight of evidence approach, taking into consideration the onset and duration of the paralysis (indicating that the neurotoxic effects in the repeated dose studies do not seem more persistent than after acute exposure), the likely cause of this (and other) neurotoxic effects, and the toxicokinetic profile of 2,5-DCBME, RAC concludes that the observed neurotoxicity in the 28-day study is acute in nature, thereby not warranting classification for STOT RE.

4.7 Germ cell mutagenicity (Mutagenicity)

4.7.1 Non-human information

The mutagenic potential of 2,5-DCBME was studied in in vitro test systems using bacteria and mammalian cells and an in vivo test system using mice. The results of the mutagenicity tests of 2,5-DCBME are summarised in Table 16.

Table 16: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Bacterial reverse mutation assay, Salmonella typh. and Escherichia coli	Negative (+/- S9)		(Vértesi, 2004 ASB2007-1385)
In vitro chromosome aberration test, Chinese hamster ovary cells	Negative (+/- S9)		(Béres, 2004 ASB2007-1386)
In vitro CHO/HPRT assay, Chinese hamster ovary cells	Negative (+/- S9)		(Béres, 2004 ASB2007-1387)
In vivo Mouse Micronucleus test, CRL:NMRI BR Mice	Negative	Dose range tested: 100-1000 µg/kg bw	(Béres, 2004 ASB2007-1388)

4.7.1.1 In vitro data

2,5-DCBME was negative in the bacterial reverse mutation assay using histidine-requiring auxotroph strains of Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537 strains) and the tryptophan-requiring auxotroph strain of Escherichia coli (WP2, uvrA strain) (Vértesi, 2004 ASB2007-1385). No clastogenic effects were seen in an in vitro chromosome aberration test using chinese hamster ovary cells (Béres, 2004 ASB2007-1386). 2,5-DCBME did not induce mutagenic effects in the CHO-HPRT forward mutation assay (Béres, 2004 ASB2007-1387).

4.7.1.2 In vivo data

No mutagenic effects were induced in the mouse micronucleus test in vivo (Béres, 2004 ASB2007-1388).

4.7.2 Human information

No relevant information is available.

4.7.3 Other relevant information

No other relevant information is available.

4.7.4 Summary and discussion of mutagenicity

Dossier submitter

2,5-DCBME was devoid of any mutagenic activity in in vitro and in vivo test systems.

Comparison with criteria

The results of the in vitro as well as the in vivo studies demonstrated, that 2,5-DCBME has no mutagenic or clastogenic potential.

Conclusions on classification and labelling

Classification and labelling is not required.

RAC evaluation

During public consultation, this proposal/endpoint was not commented on.

Given that, overall, 2,5-DCBME tested negative in three in vitro studies (a bacterial mutation assay, and a mammalian gene mutation and chromosomal aberration assay) and one in vivo study (a micronucleus assay), RAC supports the conclusion of the dossier submitter that 2,5-DCBME should not be classified for mutagenicity.

4.8 Carcinogenicity

4.8.1 Non-human information

There are no relevant data.

4.8.1.1 Carcinogenicity: oral

There are no relevant data.

4.8.1.2 Carcinogenicity: inhalation

There are no relevant data.

4.8.1.3 Carcinogenicity: dermal

There are no relevant data.

4.8.2 Human information

There are no relevant data.

4.8.3 Other relevant information

There are no other relevant data.

4.8.4 Summary and discussion of carcinogenicity

Dossier submitter

There are no data relevant to the carcinogenicity classification.

Dossier submitter's comparison with criteria

There are no relevant data to compare with criteria.

Dossier submitter's conclusions on classification and labelling

No conclusion can be drawn on classification and labelling.

RAC evaluation

During public consultation, this proposal/endpoint was not commented on.

In the absence of data, RAC agrees that no conclusion can be drawn on the classification for carcinogenicity.

4.9 Toxicity for reproduction

4.9.1 Effects on fertility

4.9.1.1 Non-human information

There are no relevant data.

4.9.1.2 Human information

There are no relevant data.

4.9.2 Developmental toxicity

4.9.2.1 Non-human information

There are no relevant data.

4.9.2.2 Human information

There are no relevant data.

4.9.3 Other relevant information

There are no other relevant data.

4.9.4 Summary and discussion of reproductive toxicity

Dossier submitter

There are no relevant data to discuss.

Comparison with criteria

There are no relevant data to compare with criteria.

Conclusions on classification and labelling

No conclusion can be drawn on classification and labelling.

RAC evaluation

The evaluation by RAC relates to the proposal of the dossier submitter not to classify 2,5-DCBME for reproductive toxicity due to absence of data. During public consultation, this proposal/endpoint was not commented on.

RAC noted that in a rat oral 28-day study, oligospermia in the epididymes was observed in 5 out of 10 males dosed with 900 mg/kg bw/d. No other effects on the testes were reported. In the absence of more detailed information on e.g. the degree of the reduction in sperm concentration, it is difficult to judge whether this effect, which was apparently no longer found after a 6-week recovery period, is of toxicological significance.

In the absence of appropriate data, RAC agrees that no conclusion can be drawn on the classification for reproductive toxicity.

4.10 Other effects

4.10.1 Non-human information

4.10.1.1 Neurotoxicity

No neurotoxicity studies have been performed.

4.10.1.2 Immunotoxicity

There are no relevant data to discuss.

4.10.1.3 Specific investigations: other studies

No special invesitigations have been performed.

4.10.1.4 Human information

No information is available.

4.10.2 Summary and discussion

No data are available to discuss.

4.10.3 Comparison with criteria

There are no relevant data to compare with criteria.

4.10.4 Conclusions on classification and labelling

No conclusion can be drawn on classification and labelling.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazard assessment for Methyl 2,5-dichlorobenzoate is based on the Draft Assessment Report and Proposed Decision of Germany prepared in the context of the inclusion of Methyl 2,5-dichlorobenzoate in Annex I of Council Directive 91/414/EEC (DAR June 2007 + Final addendum June 2008; Anonymous, 2008).

5.1 Degradation

The stability of Methyl 2,5-dichlorobenzoate was studied in hydrolysis and photolysis tests in water. The photo-chemical oxidative degradation in air was also studied. The results are summarised in Table 17. Studies on the environmental fate and behaviour of Methyl 2,5-dichlorobenzoate are not available.

Table 17: Summary of relevant information on degradation

Method	Results	Remarks	Reference

Hydrolysis rate of purified active substance - EEC C7 [Test 1 and test 3]	Measured: pH 4: 231 h (50°C) pH 7: 79 h (50°C) pH 9: 1.6 h (50°C) Calculated: pH 4: 686 h (25°C) pH 7: 389 h (25°C) pH 9: 8.8 h (25°C)		Dardemann, 2006 CHE2006-1418, CHE2006-1419 Dardemann, 2007 1691654 Statem., 1691664 Amend.
Direct photolysis in water - OECD Draft Document (August 2000) Direct photolysis rate constants estimated in near surface clear natural water at latitude 50° N:	k_d: 1.160 x 10^{-3} 1/h DT_{50}: 24.9 d quantum yield: 6.017 x 10-7 (Φ_{chem}) Photolysis rate constant k_d: spring: 2.318 x 10^{-4} 1/h summer: 3.489 x 10^{-4} 1/h fall: 1.174 x 10^{-4} 1/h winter: 5.283 x 10^{-5} 1/h DT_{50}: spring: 125 d summer: 83 d fall: 246 d winter: 547 d		Lange, 2006 CPP106132
Photo-chemical oxidative degradation in air - Atkinson (AOPWIN-software version 1.90)	Atmospheric DT_{50}: 46.3 d		(Anonymous, 2008)

5.1.1 Stability

Hydrolysis

Under sterile aqueous conditions, at temperatures of 50°C, hydrolysis rates of Methyl 2,5-dichlorobenzoate were found to be 231 h, 79 h and 1.6 h at pH 4, 7 and 9, respectively. The study was performed according to Directive 67/548/EEC Annex V, Method C.7 with Methyl 2,5-dichlorobenzoate dissolved in sterile buffers at a nominal concentration of approximately 43 mg/L (Dardemann, 2006, CHE2006-1418, CHE2006-1419). In an amendment to the hydrolysis study the DT_{50} values at 25°C were calculated to be 686 h, 389 h and 8.8 h at pH 4, pH 7 and pH 9, respectively (Dardemann, 2007, 1691664). According to the statement of Dardemann (2007, 1691654) Methyl 2,5-dichloro-benzoate do not form ions but rather hydrolyses, therefore the products of hydrolyses are 2,5-dichloro-benzoic acid and methanol.

Photolysis in water

The direct photolysis rate constant k_d and the DT_{50} value of Methyl 2,5-dichlorobenzoate under laboratory conditions were determined to be 1.160 x 10^{-3} 1/h (k_d) and 24.9 days (DT_{50}), respectively. The quantum yield of the test item was 6.017 x 10-7 (Φ_{chem}). Direct photolysis rate constants and half lives for clear natural water during spring, summer, fall and winter at latitude 50° N were estimated based on the laboratory data resulting in DT_{50} values of 125 d, 83 d, 246 d and 547 d, respectively (Lange, 2006, CPP106132).

Photolysis in soil

No data are available.

Photo-oxidative degradation in air

Based on an overall OH reaction rate of 0.3463 x 10-12 cm^3/molecule-sec obtained by addition reactions to aromatic rings of Methyl 2,5-dichlorobenzoate and hydrogen abstraction, and assuming a 12-hours-day with an OH radical concentration of 1.5 x 10^6 OH radicals/cm^3, the half-life of Methyl 2,5-dichlorobenzoate in air was calculated to be 46.3 days using the AOPWIN-software version 1.90 (Frauen, 2001, LUF2002-17). The estimation was principally confirmed by a further worst case simulation performed by the reporting member state assuming a 24-hours-day with an OH radical concentration of 5 x 10^5 OH radicals/cm^3 which results in a DT_{50} value of 46.3 d (Anonymous, 2008). Therefore the potential for long range atmospheric transport is an intrinsic property of the substance.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data are available.

5.1.2.2 Screening tests

No data are available.

A study of ready biodegradability was announced in September 2007 in relation to the preparation of the Draft Assessment Report, but it has not been delivered. For precautionary reasons the substance is classified as not ready biodegradable.

5.1.2.3 Simulation tests

No data are available.

5.1.3 Summary and discussion of degradation

A study of ready biodegradability was announced in September 2007 in relation to the preparation of the Draft Assessment Report, but it has not been delivered. For precautionary reasons the substance is classified as not ready biodegradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

No data are available.

5.2.2 Volatilisation

The vapour pressure of Methyl 2,5-dichlorobenzoate was determined using MINIVAP automatic vapour pressure tester according to the EEC Directive 92/69 Annex V Part A.4 (1992) resulting in 0.32 kPa at 20 °C and 0.37 kPa at 25 °C, respectively (Kiss, 2006, 06/148-323AN). Therefore Methyl 2,5-dichlorobenzoate was determined to be a highly volatile compound.

5.2.3 Distribution modelling

No data are available.

5.3 Aquatic Bioaccumulation

No data are available.

5.4 Aquatic toxicity

The acute toxicity to aquatic organisms was studied on fish, daphnia and algae. All submitted studies were evaluated as not valid in the Draft Assessment Report and Proposed Decision of Germany prepared in the context of the inclusion of Methyl 2,5-dichlorobenzoate in Annex I of Council Directive 91/414/EEC (DAR June 2007 + Final addendum June 2008; Anonymous, 2008). The data however reveals acute toxicity of the substance in a relevant range for classification and labelling according to Regulation (EC) No 1272/2008. Therefore a classification and labelling based on the available data is proposed.

Table 18: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Fish – acute toxicity to *Brachydanio rerio* - OECD Guideline for Testing of Chemicals No. 203	$EC_{50(96h)}$: 30.66 mg/L (nom)	Not valid	(Anonymous, 2008)
Invertebrates – acute toxicity to *Daphnia magna* - OECD Guideline for Testing of Chemicals No. 202	$EC_{50(48h)}$: 7.5 mg/L (nom)	Not valid	(Anonymous, 2008)
Algae – acute toxicity to *Pseudokirchneriella subcapitata* - OECD Guideline for Testing of Chemicals No. 201	$E_yC_{50(72h)}$: 10.49 mg/L $E_rC_{50(72h)}$: 12.53 mg/L NOEC: 1.4 mg/L	Not valid	(Anonymous, 2008)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

To determine the acute toxicity of Methyl 2,5-dichlorobenzoate (purity 99.25 % w/w, specification: batch No.: 370103) to zebra danio the test substance was weighed individually, ultrasonicated for 30 minutes in 1 L of the exposure medium and was transferred and mixed in the respective aquaria to the required concentrations. Ten fish were exposed to 8, 14, 23, 39 and 67 mg/L (nom) in whole glass aquaria (10 L) for 96 hours under static conditions. The real measured concentrations at the beginning of the test (0 hours) were 7.62, 22.53 and 65.72 mg/L, respectively. After 96 hours the active substance was not detectable. Control group was maintained with ten fish in water for 96 hours. The environmental conditions were: pH 7.75 – 8.6 temperature 21.2 – 21.6 °C, dissolved oxygen concentration 69 – 89 % and a photoperiod of 12 h dark and 12 h light. After approximately 24, 48 and 96 hours the fish in each test vessel were observed for mortality and adverse effects (behaviour and appearance). Fish were considered dead if they produced no reactions when touched on the caudal peduncle. LC_{50}

(50% lethal concentration) for test substance was estimated at 24, 48, 72 and 96 hours after exposure by cumulative mortality. LC_{50} for reference substance was determined by Finney`s probit analysis (Finney, 1971) using a software NCSS (2000). Based upon the above mortality data, the LC_{50} of Methyl 2,5-dichlorobenzoate at 24, 48, 72 and 96 h were determined as 43.12, 34.27, 30.66 and 30.66 mg/L, respectively. As the test substance hydrolysed the reported LC_{50} of Methyl 2,5-dichlorobenzoate was based on the tested nominal concentrations. (Chittibabu, 2007, 1690641)

Evaluation of the study: The test substance is rapidly degraded in water presumably by hydrolysis. Therefore it was not detectable after 96 hours by the described analytical method. The concentration of the test substance in the different test vessels was measured only twice (after 0 and 96 hours) and only for three of the five concentrations. No LOQ and LOD of the analytical test method were given. Therefore it is not possible to calculate reasonable LC_{50} values based on real concentrations according to the OECD guidance document No. 23. In general, the wrong test system was chosen in this case. For substances being rapidly degraded a flow through test or at least a semi static test is appropriate. Overall the submitted study is considered to be not valid. (Anonymous, 2008)

However, for classification and labelling all existing data should be evaluated. Hence, for the purpose of classification and labelling the study results can be used, because no other data is available for this endpoint and the use of nominal effect concentrations represents a minimum classification of the substance.

5.4.1.2 Long-term toxicity to fish

No data are available.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

To determine the acute toxicity of Methyl 2,5-dichlorobenzoate (purity 99,25 % w/w, specification: batch No.: 370103) to Daphnia magna, the test substance was diluted with dilution water to prepare a stock solution and ultrasonicated for 30 minutes. From this stock, appropriate volumes were transferred to the exposure medium to obtain the required concentration. Test organisms were exposed over a period of 48 hours. The test was performed in glass vessels under static conditions. Per test substance concentration four replicates (= for glass vessels) were set up with each test vessel containing five daphnids. The nominal test substance concentrations were 0.0 (control), 2, 3.6, 6.5, 11.7 and 21 mg/L. The real measured concentrations at the beginning of the test (0 hours) were 1.86, 6.37 and 20.61 mg/L, respectively. After 48 hours the active substance was not detectable. The environmental conditions were: pH 7.0 - 8.3, total hardness as ($CaCO_3$) 246 mg/L, conductivity 653 – 696 µS/cm, temperature 19.3 – 20.1 °C, dissolved oxygen concentration 81.4 – 96.3 % and a photoperiod of 8 h dark and 16 h light. Immobilisation was recorded after 48 h and the concentration immobilising 50 % of daphnids (EC_{50}) was calculated by Finney`s Probit analysis. The 48 hours EC_{50} Methyl 2,5-dichlorobenzoate for Daphnia magna was calculated as 7.5 mg/L (nom) and the fiducial limits to EC_{50} were calculated as 6.63 to 8.37 mg/L. As the test substance hydrolysed, the reported EC_{50} of Methyl 2,5-dichlorobenzoate was based on tested nominal concentrations. (Gopi, 2007, 1690980)

Evaluation of the study: The test substance is rapidly degraded in water presumably by hydrolysis. Therefore it was not detectable after 48 hours by the described analytical method. The concentration of the test substance in the different test vessels was measured only twice (after 0 and 48 hours) and only for three of the five concentrations. No LOQ and LOD of the analytical test method were given. Therefore it is not possible to calculate reasonable EC50 values based on real concentrations according to the OECD guidance document No. 23. In general, the wrong test system was chosen in this case. For substances being rapidly degraded

a flow through test or at least a semi static test is appropriate according to the OECD guidance document No. 23. Overall the submitted study is considered to be not valid. (Anonymous, 2008)

However, for classification and labelling all existing data should be evaluated. Hence, for the purpose of classification and labelling the study results can be used, because no other data is available for this endpoint and the use of nominal effect concentrations represents a minimum classification of the substance.

5.4.2.2 Long-term toxicity to aquatic invertebrates

No data are available.

5.4.3 Algae and aquatic plants

To determine the acute toxicity of Methyl 2,5-dichlorobenzoate (purity 99.25 % w/w, specification: batch No.: 370103) to green alga Pseudokirchneriella subcapitata (alga stock culture maintained at IIBAT, primary culture supplied by Marinco Bioassay laboratory, USA), 20 mg of the test substance was mixed with 250 mL of OECD medium (TG 201) and kept under ultra sonicator for 30 min in order to prepare a stock solution (80 mg/L). After pH adjustment the test substance stock solution was diluted with OECD medium (TG 201) and transferred into sterile 250 mL Erlenmeyer flasks with a final volume of 100 mL per replicate. The study was conducted at 0.0 (control), 0.8, 1.4, 2.6, 4.6, 8.3, and 15 mg/L (nominal) of Methyl 2,5-dichlorobenzoate in test medium with three replications per test concentration and six replications for control. The control and treated flasks were inoculated with P. subcapitata from pre-culture to obtain an initial cell concentration of 1 x 104 cells/mL. The test organisms were exposed over a period of 72 hours. The environmental conditions tests were: The inoculated flasks were kept in a shaker incubator and maintained with continuous illumination of 6930 - 7450 lux light intensity at 22.0 - 23.1 °C and pH 7.37 and 8.09. The cell counts of P. subcapitata (cells/mL) were visually counted using an Improved Neubaur`s Haemocytometer at 24, 48 and 72 hours after inoculation. The concentrations of test substance inhibiting the growth and the resulting EyC_{50} and ErC_{50} were determined using regression equations. The EyC_{50} was calculated using the regression equation of lnY = a+b lnX (Y = percent growth inhibition, X = natural log of the concentration of Methyl 2,5-dichlorobenzoate (mg/L), (a = intercept, b = slope). The ErC_{50} was calculated using the regression equation of lnY = a+b ln X (Y = log percent inhibition, X = natural log of the concentration of Methyl 2,5-dichlorobenzoate (mg/L), (a = intercept, b = slope). The NOEC was determined by Duncan's Multiple Comparison Test.

The final cell count in the control flasks at 72 hours was 960000 cells/mL. The cells of *Pseudokirchneriella subcapitata* were increased by approx. 96 times at the end of 72 hours. The nominal 0.8, 4.6, 8.3 and 15 mg/L test concentrations were verified at the initiation and completion of the test. The measured test concentrations at test initiation were 0.77, 4.52, 14.84 mg/L, respectively. The measured test concentrations at test completion after 72 hours were found to be not detectable. The maximum growth inhibition of yield was 99.65 % at 15.0 mg/L of Methyl 2,5-dichlorobenzoate and the minimum was 0.35 % at 0.8 mg/L of Methyl 2,5-dichlorobenzoate. The EyC_{50} (0-72 h) calculated using regression analysis (log concentration vs percent growth inhibition) was 10.49 mg/L. Maximum percent inhibition of specific growth rate was 93.70 % at 15.0 mg/L of Methyl 2,5-dichlorobenzoate and the minimum was 0.08 % at 0.8 mg/L of. The ErC_{50} (0 – 72 h) calculated using regression analysis (log concentration vs. log percent inhibition of the specific growth rate) was 12.53 mg/L.

The results of the study showed that Methyl 2.5-dichlorobenzoate at various concentrations has inhibitory effects on yield and specific growth rate of *Pseudokirchneriella subcapitata*. The EyC_{50} (0-72 h) and the ErC_{50} (0 – 72 h) of Methyl 2,5-dichlorobenzoate were found to be 10.49 mg/L and 12.53 mg/L, respectively. The NOEC of Methyl 2,5-dichlorobenzoate was calculated as 1.4 mg/L for yield and growth rate. (Ayyappan, 2007, 1690981)

Evaluation of the study: The test substance was not detectable after 72 hours by the described analytical method. This is probably caused by hydrolysis of the substance. As the Henrys law constant of Methyl 2,5-dichlorobenzoate is 1.63 Pa*m^3/mol and the Erlenmeyer flasks are shaken during the algae study losses due to volatilisation may become significant (for substances with Henry's law constants of 1-10 Pa*m3/mol) according the OECD guidance document No. 23. The concentration of the test substance in the different test vessels was measured only twice (after 0 and 72 hours) and only for three of the five concentrations. No LOQ and LOD of the analytical test method were given. Therefore it is not possible to calculate reasonable LC_{50} values based on real concentrations according to the OECD guidance document No. 23. The statistical determination of EC_{50} and NOEC values was not conducted according the recommendations of the OECD Guideline No. 201 (Probit-/Weibull – Analysis and Dunett's/Williams, respectively). Additionally the information about the 95 % confidence interval was not mentioned. Overall the submitted study is considered to be not valid. (Anonymous, 2008)

However, for classification and labelling all existing data should be evaluated. Hence, for the purpose of classification and labelling the study results can be used, because no other data is available for this endpoint and the use of nominal effect concentrations represents a minimum classification of the substance.

5.4.4 Other aquatic organisms (including sediment)

No data are available.

5.5 Summary and discussion of environmental hazards (sections 5.1 – 5.4)

Dossier submitter

In aquatic toxicity studies, an acute EC_{50} value for aquatic invertebrates was obtained at a nominal 2,5-DCBME concentration of 7.5 mg/l. The actual concentration of test substance over the test duration was not determined. There are no results of long-term toxicity studies for algae, invertebrates, fish and sediment dwelling organisms.

There are no data (screening or simulation tests) to assess whether 2,5-DCBME is readily biodegradable or not. Considering the results of hydrolysis and photolysis, 2,5-DCBME is considered not rapidly biodegradable (i.e. does not meet the criterion of >70% degradation within 28 days) for the purposes of classification and labelling.

2,5-DCBME has a log Kow of 3.46. There are no experimentally derived BCF values. The log Kow is above the trigger of 3 (criterion for bioaccumulating potential according to DSD), but is not above the trigger of 4 (criterion for bioaccumulating potential according to CLP).

Dossier submitter's conclusion on classification and labelling according to CLP
Methyl 2,5-dichlorobenzoate fulfils the criteria for classification as aquatic environmental hazard chronic category 2, H411 based on the lowest nominal acute toxicity data for Daphnia magna (EC_{50} = 7.5 mg/l) in a 48-h static study.

Dossier submitter's conclusions on classification and labelling according to DSD
Methyl 2,5-dichlorobenzoate fulfils the criteria for classification with N; R51-53.

Based on the lowest nominal toxicity data for *Daphnia magna* (EC50 = 7.5 mg/l) in a 48-h static study the following specific concentration limits should be applied:

Concentration	Classification
C ≥ 25%	N; R51-53
2.5% ≤ C < 25%	R52-53

where C is the concentration of Methyl 2,5-dichlorobenzoate in the preparation

RAC evaluation

The evaluation by RAC relates to the proposal of the dossier submitter to classify 2,5-DCBME for aquatic chronic toxicity. Originally, the dossier submitter proposed to classify the substance for both aquatic acute and aquatic chronic toxicity (with Aquatic Acute 1 – H400, Aquatic Chronic 1 – H410, M-factor 1 (CLP) and N; R50-53 with corresponding concentration limits (DSD)). This original proposal was commented on during public consultation. Disagreement with the proposed precautionary classification based on the absence of reliable data was expressed by some parties and classification was considered inappropriate. Others supported the precautionary classification or expressed sympathy with the need to classify based on available data but recommended that supporting data be added to strengthen the proposal.

In response to these comments, the dossier submitter amended the classification proposal to a (downgraded) classification for aquatic chronic toxicity (with Aquatic Chronic 2 – H411 (CLP) and N; R51-53 (DSD)).

Limited data are available on the degradability of 2,5-DCBME. At pH 4 and pH 7, 2,5-DCBME hydrolysis is slow with DT_{50} values of 686 and 389 hours, respectively, but at pH 9 the hydrolysis is faster with a DT_{50} of 8.8 hours. The expected primary hydrolysis products are 2,5-dichlorobenzoic acid and methanol. The presence of these products was not monitored in the hydrolysis study. Methyl 2,5-dichlorobenzoate undergoes slow photolysis in water with calculated DT_{50} values of 83-547 days at latitude 50 °N. No information is available on the biodegradability of 2,5-DCBME. No information on the degradation or toxicity of the breakdown products is presented. Methyl 2,5-dichlorobenzoate must be considered as not rapidly degradable (CLP) and not readily degradable (DSD) for the purpose of classification and labelling as it does not degrade biotically or abiotically in the aquatic environment to a level > 70% within a 28-day period and the available data do not demonstrate that the breakdown products are classifiable.

Methyl 2,5-dichlorobenzoate has a log Kow of 3.46. No measured BCF values are available.

The acute aquatic toxicity of 2,5-DCBME has been assessed in fish, crustaceans and algae. The $LE(C)_{50}$ obtained in fish, crustaceans (Daphnia) and algae were 30.66 mg/l, 7.5 mg/l and 12.5 mg/l, respectively, based on nominal concentrations. Chronic toxicity values are only available for algae with a NOEC of 1.4 mg/l, based on a nominal concentration. However, due to the rapid decline of test substance concentrations in the medium, attributed to hydrolysis and/or volatilization, the nominal concentrations do not reflect the actual exposure concentrations. The test substance concentrations were only measured at the beginning of the study and at termination; at test termination, no 2,5-DCBME could be detected in any of the studies. The actual toxicity of 2,5-DCBME could therefore have been underestimated.

Given the nominal 48 h EC_{50} of 7.5 mg/l for Daphnia, and the fact that 2,5-DCBME must be considered as not rapidly/readily degradable, the criteria for classification for aquatic chronic toxicity, category 2 (for $L(E)C_{50}$ values between 1 and 10 mg/l) are met (Aquatic Chronic 2 – H411 (CLP); N; R51-53 (DSD). This should be considered as a minimum classification. As the currently available experimental data for 2,5-DCBME do not enable another classification to be considered, RAC did some QSAR predictions and analysed some data on structurally similar substances have been investigated, in order to establish whether another classification is more appropriate for 2,5-DCBME. Given the limitations for RAC elaborations to go beyond the information provided in the dossier and during public consultation, it should be noted that this additional work by RAC was clearly more limited than the thorough and structured QSAR and read across analysis that would be expected from a dossier submitter for effective decision support.

The QSAR program ECOSAR (v1.00) was used to predict the aquatic toxicity of 2,5-DCBME, using the model for esters (valid for a.o. benzoates where the log Kow is below the range 5-8 and (for solids) the $L(E)C_{50}$/NOECD does not exceed the water solubility). The measured log Kow value (3.46), melting point (34.6°C) and water solubility (87 mg/l) were used in the calculations. The following results were obtained:

Fish 96-h LC_{50} = 4.1 mg/l
Daphnid 48-h LC_{50} = 6.9 mg/l
Mysid shrimp 96-h LC_{50} = 2.7 mg/l
Green algae 96-h EC_{50} = 2.5 mg/l

In an analysis of substances that are structurally similar to 2,5-DCBME (ECHA, 2012,), it was found that substances similar to 2,5-DCBME often have a harmonised classification for environmental hazards. However, some are not classified, indicating that aquatic toxicity may be very sensitive to the molecular structure. It would therefore generally be preferable to rely on data on the specific substance rather than attempting a read-across. Nevertheless, a structure-activity analysis was attempted, based on the assumption that the substitution pattern of the phenyl-ring influences the overall reactivity, the reaction rate being faster the more electron-withdrawing groups are present on the aromatic ring. The phenyl-ring in 2,5-DCBME is connected to two chlorine atoms, which are electron-withdrawing groups, and to one methylester-group, which is also electron-withdrawing when connected as $–C(=O)OCH_3$.

Three substances were used for read across. The first substance has a phenyl-ring with one halogen and a methylester-group (connected as $–OC(=O)CH_3$) attached. When connected in this way, the ester-group is electron-donating. This substance is classified as Aquatic Chronic 2. Compared to this substance, the reactivity of 2,5-DCBME is expected to be higher, because 2,5-DCBME has more (and stronger) electron-withdrawing groups and no electron-donating group. With an expected higher reactivity, leading to a higher toxicity, 2,5-DCBME should thus also be classified for aquatic toxicity.

The second substance also has a phenyl-ring connected to a methylester-group and to one halogen, but in this case the methylester is connected as in 2,5-DCBME. Additionally, this substance has an electron-donating amine group connected to the phenyl-ring. It is classified as Aquatic Chronic 3. Compared to this substance, the reactivity of 2,5-DCBME is again expected to be higher, because 2,5-DCBME has more (and stronger) electron-withdrawing groups and no electron-donating group. Consequently, 2,5-DCBME should also be classified.

In a third substance (50% pure) used for read-across, which is classified as Aquatic Chronic 3, the phenyl-ring is connected to two chlorine atoms and to both an acid- and a methoxy-group. The latter group is electron-donating, whereas the acid-group is electron-withdrawing. Given that 2,5-DCBME has no electron-donating group, its reactivity is expected to be higher and thus it should also be classified.

All in all, the QSAR predictions and the analysis of structurally similar substances are considered to substantiate the need for classification for aquatic toxicity, but they are not considered sufficient to judge whether a more stringent classification than the minimum classification is necessary.

Based on all available information, RAC supports the conclusion of the dossier submitter that 2,5-DCBME should be classified for aquatic chronic toxicity with **Aquatic Chronic 2 – H411** (CLP) and **N; R51-53**, (DSD, no specific concentration limits necessary). The classification may need to be reviewed if any valid aquatic toxicity data become available.

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