Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

carvone

EC number: 202-759-5 (d/l mixture of stereoisomers)

218-827-2 (d-carvone)

229-352-5 (l-carvone)

CAS number: 99-49-0 (d/l mixture of stereoisomers)

2244-16-8 (d-carvone)

6485-40-1 (l-carvone)

CLH-O-0000003038-78-03/F

Adopted

4 June 2013

4 June 2013

CLH-O-0000003038-78-03/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 (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: carvone

EC numbers: 202-759-5 (d/l mixture of stereoisomers)

218-827-2 (d-carvone)

229-352-5 (l-carvone)

CAS numbers: 99-49-0 (d/l mixture of stereoisomers)

2244-16-8 (d-carvone)

6485-40-1 (l-carvone)

The proposal was submitted by **the Netherlands** and received by the RAC on **23 October 2012.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands 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 **23 October 2012**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 December 2012**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Norbert Rupprich**

Co-rapporteur, appointed by RAC: **Pietro Paris**

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

The RAC opinion on the proposed harmonised classification and labelling was reached on **4 June 2013** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**

OPINION OF THE RAC

The RAC adopted the opinion that carvone and its stereoisomers (alone or as a mixture) should be classified and labelled as follows:

Classification and labelling in accordance with the CLP

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard state-ment Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code	Suppl. Hazard state-ment Code(s)	
Current Annex VI entry	No current entry									
Dossier submitters proposal	606-148-00-8	carvone (ISO); 2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [1] d-carvone; (5S)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [2] l-carvone; (5R)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one [3]	202-759-5 [1] 218-827-2 [2] 229-352-5 [3]	99-49-0 [1] 2244-16-8 [2] 6485-40-1 [3]	Skin irrit. 2 Skin sens. 1B	H315 H317	GHS07 Wng	H315 H317		
RAC opinion	606-148-00-8	carvone (ISO); 2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [1] d-carvone; (5S)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [2] l-carvone; (5R)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one [3]	202-759-5 [1] 218-827-2 [2] 229-352-5 [3]	99-49-0 [1] 2244-16-8 [2] 6485-40-1 [3]	Skin sens. 1	H317	GHS07 Wng	H317		

Resulting Annex VI entry if agreed by COM	606-148-00-8	carvone (ISO); 2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [1] d-carvone; (5S)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [2] l-carvone; (5R)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one [3]	202-759-5 [1] 218-827-2 [2] 229-352-5 [3]	99-49-0 [1] 2244-16-8 [2] 6485-40-1 [3]	Skin sens. 1	H317	GHS07 Wng	H317		

Classification and labelling in accordance with DSD

	Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**	**Labelling**	**Concentration Limits**
Current Annex VI entry	No current entry						
Dossier submitters proposal	606-148-00-8	carvone (ISO); 2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [1] d-carvone; (5S)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [2] l-carvone; (5R)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one [3]	202-759-5 [1] 218-827-2 [2] 229-352-5 [3]	99-49-0 [1] 2244-16-8 [2] 6485-40-1 [3]	Xi; R38 R43	Xi R: 38-43 S: (2-)24-37	
RAC opinion	606-148-00-8	carvone (ISO); 2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [1] d-carvone; (5S)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [2]	202-759-5 [1] 218-827-2 [2] 229-352-5 [3]	99-49-0 [1] 2244-16-8 [2] 6485-40-1 [3]	R43	Xi R: 43 S: (2-)24-37	

		l-carvone; (5R)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one [3]					
Resulting Annex VI entry if agreed by COM	606-148-00-8	carvone (ISO); 2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [1] d-carvone; (5S)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one; [2] l-carvone; (5R)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one [3]	202-759-5 [1] 218-827-2 [2] 229-352-5 [3]	99-49-0 [1] 2244-16-8 [2] 6485-40-1 [3]	R43	Xi R: 43 S: (2-)24-37	

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

Carvone is a terpenoid which is found in plants and seeds. The major natural sources of carvone are found in caraway, dill and spearmint essential oils. Carvone exists as two stereoisomers: (R)-carvone or l-carvone which has a spearmint-like odour and (S)-carvone or d-carvone, which has a caraway-like odour (de Carvalho and da Fonseca, 2005).

Both carvones are used in the food and flavor industry, in consumer products as well as a plant protection product (PPP). d-Carvone is used to prevent premature sprouting of potatoes during storage, while. l-Carvone is used as an insect repellent.

Carvone stereoisomers have no entry in Annex VI of the CLP Regulation. The proposal from the Dossier Submitter covers both stereoisomers so that they will appear as a single entry in Annex VI of CLP Regulation, e.g. similar to limonene stereoisomers. The database consists of studies performed with d-carvone, l-carvone, or carvone with a (non-)specified isomer ratio.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Based on the available acute toxicity studies the DS did not propose to classify carvone for acute oral, dermal or inhalation toxicity.

Comments received during public consultation

An industry stakeholder representative submitted an acute dermal toxicity study in rats to substantiate the lack of skin irritation potential of *l*-carvone (Sanders, 1999). In that study, the LD_{50} of *l*-Carvone (purity is 99.4%) was found to be higher than 2000 mg/kg body weight. There were no clinical signs (including no skin irritation), no mortality and no abnormalities at necropsy. Industry used these study results and referred to the REACH regulation (Annex VIII, 8.1.1) to argue against the classification of *l*-carvone as a skin irritant (see the RAC evaluation of skin corrosion/irritation).

Assessment and comparison with the classification criteria

Carvone (isomer ratio not specified) was tested at a single oral dose of 2000 mg/kg/d. There were clinical signs, but no mortality or abnormalities at necropsy. A second acute oral toxicity study was judged to be unreliable. According to DSD and CLP criteria a substance should not be classified if the oral LD_{50} is > 2000 mg/kg/d.

Acute dermal toxicity was tested with carvone (isomer ratio not specified) at a single dose of 4000 mg/kg/d. Carvone did not cause acute adverse effects (neither lethality nor systemic or dermal effects). Industry submitted another acute dermal toxicity study in rats (Sanders, 1999). In that study, the LD_{50} of *l*-Carvone (purity 99.4%) was found to be higher than 2000 mg/kg body weight. There were no clinical signs (including no skin irritation), no mortality and no abnormalities at necropsy. According to DSD and CLP criteria, a substance should not be classified if the dermal LD_{50} is > 2000 mg/kg.

Acute inhalation toxicity was tested with carvone with a d/l isomer ratio of at least 4:1 at a single dose of 5.66 g/m³. One female died on the day after the exposure. Mild to moderate clinical signs were observed during or after exposure. Body weight gain was impaired in most rats during the first week after treatment. Pathology revealed no abnormalities, except in the female that died the day after exposure. The respiratory LC_{50} of carvone in rats was >5.66 g/m3. According to the CLP criteria, substances should not be classified if the inhalation LC_{50} is greater than 5 mg/l (dusts and mists).

Overall, carvone does not meet the classification criteria (CLP and DSD) for acute toxicity (oral, dermal, by inhalation). The RAC supported the proposal of the DS not to classify carvone for acute oral, dermal or inhalation toxicity.

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

Summary of the Dossier submitter's proposal
Based on the assessment of the non-lethal adverse effects caused by carvone in the acute oral and inhalation studies, the DS did not propose a classification of carvone for specific target organ toxicity (single exposure).

Comments received during public consultation
No comments were received during public consultation.

Assessment and comparison with the classification criteria
Non-lethal adverse effects in acute toxicity testing (rats) were only observed following oral and inhalation exposure (not in the acute dermal toxicity study).

The clinical signs in the oral acute toxicity study at 2000 mg/kg included hunched posture, lethargy and body tremor. No abnormalities were seen at necropsy. These adverse effects are not considered to be "significant functional changes, more than transient in nature" (as stated in the CLP Regulation). Additionally, accounting for the high dose level tested, it is RAC's opinion that the criteria for STOT SE (category 1 and 2) are not fulfilled. Oral toxicity testing did not result in narcotic effects, thus STOT SE (category 3) is also not warranted.

Inhalation toxicity was tested at a single dose level (5.66 g/m³). One female rat died. It is not described which of the non-lethal adverse effects only occurred in the single rat that died. In general, inhalation exposure severely affected breathing patterns and in addition resulted in restlessness, stress, incoordination and tremors. Body weight gain was impaired. Pathological investigations revealed no abnormalities. RAC concluded that these health effects at the rather high dose level of 5.66 g/m³ do not fulfil the criteria for STOT SE (category 1 or 2). The respiratory effects do not necessarily indicate respiratory tract irritation; thus there is not sufficient evidence to classify carvone for STOT SE (category 3).

Overall, RAC concluded, in agreement with the DS, that classification for STOT SE is not warranted for carvone.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal
The dossier submitter (DS) proposed to classify both stereoisomers of carvone (d- and l-carvone) as skin irritants. This proposal is based on the results of a skin irritation test in rabbits with a carvone mixture in which the ratio of stereoisomers were not specified. The DS justifies this proposal with the occurrence of skin desquamation which started to develop 48 hours after application and persisted in all three animals through to the end of the observation period (7 days). Inflammation that persists to the end of the observation period (normally 14 days) in at least 2 animals is a classification criterion for skin irritation according to both Directive 67/548/EC (DSD) and Regulation (EC) 1272/2008 (CLP). Scores for erythema and oedema were up to grade 1 or 2 but did not yield the values necessary to justify the proposal for classification based on erythema and/or oedema. Because there was no information indicating that one of the stereoisomers is clearly more toxic or irritating than the other, it was the proposal of the dossier submitter to classify both stereoisomers as skin irritants.

Comments received during public consultation
Three member states (MS) supported the classification proposal.. Industry however, referred to an acute dermal toxicity study with l-carvone in rats in which detailed dermal observation did not indicate signs of dermal irritation. Industry additionally pointed out that (1) the term "desquamation" is not included in the current classification criteria for skin irritation and that (2) the relevant Reach Annex on standard information requirements allows for study waiving if an acute toxicity study by the dermal route does not indicate skin irritation up to the limit dose (2000 mg/kg).

In its response the DS clarified that one of the criteria resulting in classification for skin irritation is inflammation persisting to the end of the observation period. Persistence of skin irritation can be judged by different parameters (e.g. scaling, which is another term for desquamation). The DS further emphasised that rat skin is less sensitive to irritants than rabbit skin and that the dermal doses per surface area have been lower in the acute dermal study in rats compared to the skin irritation study in rabbit. Based on this reasoning, the DS reaffirmed the proposal to classify both stereoisomers of carvone as skin irritants.

Assessment and comparison with the classification criteria

In the standard skin irritation study in rabbits conducted with a mixture of carvone stereoisomers, the scores for erythema and/or oedema are elevated but not sufficiently to warrant classification. The only trigger for the classification proposal is the skin desquamation which was first observed in 1 animal at day 2 post application and which had developed in all 3 animals by day 7 (observation period up to 7 days). There is no information on the degree/severity of this desquamation. It is to be noted that the ECHA guidance on information requirements and chemical safety assessment (chapter R.7.a: endpoint specific guidance) for skin irritation (appendix R.7.2-1) refers to the disturbance of the desquamation process as a clinically relevant element of chronic irritant contact dermatitis (ICD).

For the purpose of the assessment of skin irritation, the RAC additionally checked other toxicological studies conducted with carvone. In addition to the rat acute dermal toxicity study with l-carvone submitted during public consultation, another rat acute dermal toxicity study is available with carvone in which the ratio of stereoisomers was not specified. In both acute dermal toxicity studies in rats there was no indication of dermal irritation. In the skin sensitisation study in guinea pigs (50% and 75% carvone, isomer ratios not specified) there was also no skin reaction. An overview of the most important findings and test conditions are presented in the table below.

Most relevant parameter	**Skin irritation test**	**Skin sensitisation test**	**2 acute dermal toxicity tests**
Species	*Rabbit*	*Guinea pig*	*Rat*
Carvone isomer ratio	Not known	Not known	Not known or l-carvone
Sensitivity of tested species to skin irritation	Relatively high compared to rats and guinea pigs	Relatively low compared to rats	Relatively low compared to rabbits
Exposure conditions	Semi-occlusive, 4h	Occlusive, 24h	Occlusive, 24h
Dose per skin surface area	80 mg/cm² Carvone without vehicle	Up to 75% carvone in arachis oil	20 mg/cm² Carvone without vehicle
Scores for erythema and/or oedema	Elevated, but not sufficient for classification	No dermal reactions	No dermal reactions
Other types of dermal reactions	Desquamation in 3 animals up to the observation period of 7 days, severity not reported	No other types of skin reactions reported	No other types of skin reactions reported

Based on the comparative skin irritation data outlined in the table above, the RAC recognises that:

- the only relevant evidence for skin irritation is the desquamation in rabbits,
- the desquamation was persistent up to the end of the observation period of 7 days,
- the severity of the desquamation was not reported,
- there were no relevant scores for erythema/oedema in any of the three species tested,
- in general the rabbit skin is more sensitive than the rat skin, and the rat skin is more sensitive than the guinea pig skin (ECHA guidance on CLP). But it has also been shown that the rabbit skin might be more sensitive to some substances than the human skin (Jirova *et al*. 2007),

- Exposure was via occlusive conditions in the less sensitive species. In the assessment, greater weight was placed on the duration of exposure and occlusive conditions of exposure and less weight on the dose per skin area.

There was no severity information available for the only relevant dermal reaction (desquamation).. Overall, there is not sufficient information on the severity and persistence of skin desquamation to justify classification. Furthermore, scores for erythema/oedema were not sufficiently high for classification in all three species tested.

In conclusion, the RAC is of the opinion) that the information provided shows that carvone and carvone stereoisomers do not meet the criteria for classification as a skin irritant.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

Based on an eye irritation study in rabbits, carvone (stereoisomers not specified) was found to be mildly irritating to the rabbit eye. Comparing the degree of eye irritation with the CLH and DSD classification criteria, the DS proposed that carvone need not be classified for eye irritation.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

With reference to the CLH report, carvone is reported to be mildly irritating to the rabbit eye. The following table is a copy of table 12 of the CLH report and provides animal-specific eye irritation scores up to 3 days after exposure.

Scores observed after	**1 hour**	**1 day**	**2 days**	**3 days**
Cornea				
degree of opacity	d, 0,0	1,0,0	1,0,0	0,0,0
area of opacity	4,0,0	2,0,0	1,0,0	0,0,0
Iris	1,1,1	1,0,0	0,0,0	0,0,0
Conjunctival redness	1,1,1	2,1,1	1,0,0	0,0,0
Conjunctival chemosis	1,1,1	1,0,0	0,0,0	0,0,0
Conjunctival discharge	2,0,0	2,0,0	0,0,0	0,0,0

d= dulling of the normal lustre of the corneal surface.

The rabbit treated without anaesthetic showed an initial pain reaction of 3 (scale not specified).

The CLP classification criteria for eye irritation are more stringent than the corresponding DSD criteria. The CLP cut-off values (time-weighted mean values) are 1 for corneal opacity and iritis, and 2 for conjunctival redness and oedema. It is evident that for all tested animals the relevant experimental scores were below the relevant cut-off levels.

In conclusion, the RAC is of the opinion (in agreement with the dossier submitter's proposal) that the information provided shows that carvone and carvone stereoisomers do not meet the criteria for classification as an eye irritant.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The DS proposed that carvone should be classified as a skin sensitiser (sub-category 1B). This proposal is mainly based on the results of a Guinea-pig Maximisation Test (GPMT) study.

Comments received during public consultation

The three member states (MS) supported the proposed classification of carvone as a skin sensitiser.

During public consultation one member state provided additional references relevant to the skin sensitising potential of d- and l-carvone. One of these references (Nilsson *et al*., 2001) shows that both stereoisomers of carvone are sensitising in guinea pigs. Both d-carvone (S-Carvone) and l-carvone (R-carvone) were tested for skin sensitisation according to the Freund's complete adjuvant test (FCAT).

In the Nilsson *et al* (2001) study there is the additional information that patch test responses qualify R-carvone (and possibly S-carvone) as a human skin sensitiser. The experimental study results are summarised in the following table.

Guinea pig	**Induction**	**Challenge**		
Dose groups			**Erythema**	
	Intradermal		48 h after application	72 h after application
Control	-	1% carvone	0/15	0/15
d-carvone (S-carvone)	5%	1% carvone	11/15	13/15
l-carvone (R-carvone)	5%	1% carvone	13/15	15/15

Assessment and comparison with the classification criteria

The classification proposal is mainly based on the results of a skin sensitisation GPMT study. The relevant results are summarised in the following table. The carvone stereoisomers tested are not specified.

	Induction		**Challenge**		
				Erythema / Desquamation	
	Intradermal	**Topical**	**Topical**	**24 h**	**48 h**
Control	-	-	75% carvone	0/10	0/10
Test group 1	5%	Undiluted	75% carvone	9/19 47%	1/19 Desquamation:3/19
Test group 2	5%	Undiluted	50% carvone	10/19 53%	1/19 Desquamation:2/19

For carvone, the classification criteria for skin sensitisation are fulfilled both under DSD (R43) and CLP (sensitisation 1) since more than 30% of the tested animals showed a positive response.

The proposal for classification for skin sensitisation is further strengthened by the results of the Nilsson study (2001). Furthermore, these results indicate that the skin sensitisation potential of both stereoisomers (d- and l-carvone) can be considered rather similar.

Based on these data RAC concluded that Carvone (and its stereoisomers) be classified as a skin sensitiser 1 (H317). This conclusion is in agreement with the DS's proposal (except for the sub-categorisation) and the comments received during public consultation.

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

Based on the findings in the repeated dose toxicity studies (rats and mice) the DS did not recognise the need to classify carvone for specific target organ toxicity (repeated exposure).

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Carvone was tested for repeated dose toxicity in various rat and mouse oral toxicity studies. The following table contains summaries of the study-specific experimental findings. Comparison with the classification criteria (CLP and DSD) indicates that a classification for carvone for repeated dose toxicity is not warranted. Based on a comparison of findings only in the 90-day studies, the rat is considered more sensitive to carvone than the mouse.

In the 90-day rat study the LOAEL for male and female rats is lower than the cut-off levels for STOT RE 2 respectively R48/22. However, nephropathy in male rats (accumulation of a2u-globulin) is considered to be a rat-specific health effect of no human relevance. The adverse effects noted in female rats are not considered to be severe damage, in particular because of the absence of associated histopathological changes.

Cut-off levels and dose-response data for oral repeated dose toxicity studies (in mg/kg/d)						
	R48 /25	**STOT RE 1**	**R48 /22**	**STOT RE 2**	**Dose-response data for repeated dose toxicity studies**	**CL Pro-posal**
Rat 14 d	15	30	150	300	Ratio d/l unspecified Doses: 0, 50, 200, 1000 mg/kg/d 1000 mg/kg/d: 100% mortality, forestomach effects in dead animals LOAEL of 200 mg/kg/d: slight effects on haematological and biochemical parameters, kidney weight of males (absolute and relative) significantly increased NOAEL of 50 mg/kg/d DS does not specify an "effective dose". Based on the data available in the CLH report, 200 mg/kg/d is considered a LOAEL, but not an "effective dose". Thus there is no direct experimental evidence that the effective dose is less than the highest cut-off level of 300 mg/kg/d.	no
Mouse 16 d	15	30	150	300	d-carvone Doses: 0, 150, 328, 723, 1590, 3000 mg/kg/d 1590 mg/kg/d and above: 100% mortality 723 mg/kg/d and above: dose-related increase in the incidence of clinical signs 150 mg/kg/d and above: relative liver weights increased and thymus weights decreased DS does not specify an "effective dose". Based on the data available in the CLH report, 150 mg/kg/d is considered a LOAEL, but not an "effective dose". Based on the adverse effects observed up to the dose level of 328 mg/kg/d (organ weight changes) there is no experimental evidence that the effective dose is less than the highest cut-off level of 300 mg/kg/d.	no

Rat 90 d	5	10	50	100	d/l ratio unspecified Doses: 0, 5, 30, 180 mg/kg/d NOAEL of 5 mg/kg/d <u>Males</u> At 30 mg/kg/d and above: enlarged kidneys and tubuluar necrosis. Severe hyalin droplets within the proximal tubular cells. Positive staining with antibody against a2µ-globulin. The LOAEL of 30 mg/kg/d for male rats is lower than the cut-off criteria for R48/22 and STOT RE 2. However, it is concluded that the renal histopatological changes are the result of accumulation of a2u-globulin, a MOA not considered relevant for humans. Thus the kidney effects in male rats do not warrant classification. <u>Females</u> At 30 mg/kg/d and above: Increases in partial thromboplastine time (PTT) and liver and kidney weights, and decreases in serum albumin, Ca and thymus weight. The LOAEL of 30 mg/kg/d for female rats is lower than the cut-off criteria for R48/22 and STOT RE 2. It is concluded that although the adverse effects in females have to be considered toxicologically relevant they should not considered to be serious damage (no histopathological changes in liver and kidney). Thus the adverse effects in female rats do not warrant classification.	no
Mouse 90 d	5	10	50	100	d-carvone Doses: 0, 93, 187, 375, 750, 1500 mg/kg/d Mortality and clinical signs at 1500 mg/kg/d Relative liver weight increased at 750 mg/kg/d NOAEL 375 mg/kg/d No haematological or biochemical analysis. Conclusion: the NOAEL is higher than the highest cut-off level of 300 mg/kg/d.	no

Rat 16 w	5	10	50	100	Test material: ? Dose: 500 mg/kg/d At this dose level: growth retardation and testicular atrophy According to the DS the study showed major shortcomings Dose level of 500 mg/kg/d higher than the highest cut-off level of 100 mg/kg/d	no
Rat 28 w	2.5	5	25	50	Test material: ? Dose: 50 mg/kg/d (no effects) According to the DS the study showed major shortcomings	no
Rat 1 y	1,25	2,5	12,5	25	Dose 125 mg/kg/d (no effects) According to the DS the study showed major shortcomings	no
Mouse 2 y	0.625	1.25	6.25	12.5	d-carvone Doses: 0, 375, 750 mg/kg/d (carcinogenicity study) Various dose-related effects were seen in histopathological investigations, especially in females. However, the lowest dose tested was very much higher than the highest cut-off level for classification.	no

In conclusion, RAC is of the opinion (in agreement with the dossier submitter's proposal) that the information provided shows that carvone and carvone stereoisomers do not meet the criteria for classification for specific target organ toxicity (repeated exposure).

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Based on the results of *in vitro* and *in vivo* mutagenicity studies, the DS did not consider carvone to be a genotoxic substance. The DS proposed not to classify carvone for germ cell mutagenicity.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The following table contains a summary of the available mutagenicity data. It is a well-recognised principle of assessment of mutagenicity data that in vivo findings generally overrule corresponding in vitro findings. Regarding DNA-damage, there were positive results from in vitro testing (SCE in CHO cells) while results from in vivo testing (UDS in liver) were negative. There were no positive findings of genetic mutations from in vitro testing. The results of in vitro testing for chromosome aberrations were not clear-cut, while in vivo testing (micronucleus test) results were negative. Thus, overall, these findings indicate that carvone should not be considered a genotoxic agent.

	DNA damage	Gene mutation	Chromosome aberration
In vitro	Sister chromatid exchanges in CHO cells: **positive**	Ames test (1): **negative** Ames test (2): **negative** Gene mutation in mouse lymphoma cells: **equivocal**	Chromosome aberrations in human lymphocytes: **positive (-S9)**, **negative (+S9)** Chromosome aberrations in CHO cells: **equivocal**
In vivo	In vivo UDS assay in liver: **negative**		In vivo micronucleus test: **negative**

In conclusion, the RAC is of the opinion (in agreement with the dossier submitter's proposal) that the information provided shows that carvone and carvone stereoisomers do not meet the criteria for classification for mutagenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

Based on the results of the carcinogenicity study in mice (oral gavage), the DS concluded that for d-carvone there was no evidence of carcinogenic activity. The DS expressed that there is no need to classify carvone for carcinogenicity.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

There is only one carcinogenicity study available (d-carvone, oral gavage, mice). Dose levels tested (375 and 750 mg/kg/d) resulted in various dose-related non-neoplastic effects being observed at histopathological investigations (especially in females). The DS reported that carvone did not cause clinical signs or a relevant reduction of body weight gain.

Male mice

The DS concluded that in males no increase of any type of tumour was observed. The following table contains the male-related tumour data from table 21 in the CLH report: From this table it is evident that carvone did not cause increased tumour incidences in male mice. Thus the RAC agrees with the DS's assessment that exposure to carvone did not result in evidence of carcinogenic potential in male mice.

No comments were received during public consultation.

Summary of neoplastic findings in male mice	Control	375 mg/kg/d	750 mg/kg/d
Liver (n = 50, 50, 49)			
Hepatocellular carcinoma	5	3	3
Hepatocellular adenoma	2	4	4
Lymphoma malignant mixed	2	0	1
Spleen (n = 50, 50, 48)			
Lymphoma malignant mixed	2	1	1

Skin (n = 50, 50, 50)			
Back, subcutaneous tissue, fibroma	2	0	0
Subcutaneous tissue, neurofibrosarcoma	2	1	0
Subcutaneous tissue, sarcoma	4	0	2
Lung (n = 50, 50, 50)			
Alveolar/bronchiolar adenoma	7	4	5
Harderian gland (n = 50, 50, 50)			
Adenoma	1	2	1
Multiple organs (n = 50, 50, 50)			
Hemangioma	2	0	0
Lymphoma malignant mixed	4	1	1

Note: Only neoplasms with an incidence of >1 per organ and dose are included in the table

Female mice

In female mice the overall incidence of neoplasms (specifically, mixed malignant lymphoma) was slightly higher in the treated groups (please see the following table). The DS considered it likely that this may be related to unusually low tumour incidences in the female control group due to the high early mortality rate in this control group. The DS stated that "the increased mortality in the females of the control group is most likely caused by an increased incidence of abscesses of the ovary and uterus possibly as a result from infection." Moreover the DS expressed that there was no difference in neoplastic incidences between the two treatment groups, indicating the absence of a dose-response relationship. Overall, the DS concluded that in female mice there also was no evidence of substance-related carcinogenic activity of d-carvone.

RAC acknowledges the relatively high mortality rate in female mice. However, RAC notes that the mean survival (in days) of control animals is not that much lower than in treated groups in order to convincingly explain the relatively low tumour incidences in the controls. RAC however cannot analyse the influence of reduced mean survival in depth because there is no specific information available (e.g. on the latency of mixed malignant lymphomas).

The table below presents the mortality and mean survival rate in female mice treated with carvone.

Mortality and mean survival in female mice

Dose (mg/kg/d)	0	375	750
Mortality	36/50	21/50	12/50
Mean survival (days)	639	652	676

From the following table on tumour incidences in female mice it is evident that mixed malignant lymphoma is the tumour type which needs to be specifically evaluated. Therefore RAC had a closer look at the corresponding historical control data.

Malignant lymphomas are among the most common tumours in female B6C3F1 mice. The mean historical control rate of malignant lymphomas (all sites, about 1000 animals tested) is reported to be about 20%, with a range of neoplasm rates of about 6 to 40 % among control groups. The corresponding NTP historical control database consists of all studies carried out within a time window of approximately 7 years (up to January 1997).

When compared with the neoplasm rates in untreated controls a decade before (up to about 1987) there has only been a slight decrease in the control incidences (from 27% to about 21%). Because of these relatively stable historical control rates for malignant lymphomas in female B6C3F1 mice, these data are considered to provide a valid comparison with the incidences of malignant lymphomas in the carvone carcinogenicity study. The highest incidence for mixed malignant lymphoma in treated female mice is 4/50; thus the highest incidence is below 10%. This incidence is clearly below the mean historical control rate of about 20% and similar in magnitude to the lowest study-specific historical control incidences reported (Ward, 2006; Haseman *et al*. 1998).

Summary of neoplastic findings in female mice	**Control**	**375 mg/kg/d**	**750 mg/kg/d**
Liver (50, 50, 50)			
Hepatocellular carcinoma	0	2	1
Hepatocellular adenoma	1	0	0
Lymphoma malignant mixed	0	3	3
Stomach forestomach (47, 47, 49)			
Papilloma squamous	0	3	0
Uterus (50, 50, 50)			
Polyp	0	0	2
Lymph node, mesenteric (46, 47, 48)			
Lymphoma malignant mixed	0	3	2
Spleen (50, 49, 50)			
Lymphoma malignant mixed	1	4	4
Lung (50, 50, 50)			
Alveolar/bronchiolar adenoma	1	6	3
Harderian gland (50, 50, 50)			
Adenoma	2	0	0
Kidney (50, 50, 50)			
Lymphoma malignant mixed	0	1	2
Multiple organs (50, 50, 50)			
Lymphoma malignant mixed	1	4	4

Note: Only neoplasms with an incidence of >1 per organ and dose are included in the table

Overall, the RAC concludes that there is no carcinogenic potential of carvone in male mice. For female mice, the RAC supports the analysis of the DS that the overall incidence of neoplasms (specifically, mixed malignant lymphoma) in the treated female groups is slightly elevated.
There might be a relatively low tumour incidence in the controls because of a relatively high mortality in the controls. Additional comparison of tumour rates of mixed malignant lymphoma in treated groups with relevant historical control incidences support the interpretation that tumour incidences observed in treated groups should not be considered treatment-related (Ward, 2006; Haseman *et al*. 1998).

In conclusion, the RAC is of the opinion (in agreement with the dossier submitter's proposal) that the information provided shows that carvone and carvone stereoisomers do not meet the criteria for classification for carcinogenicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Based on the results of a teratogenicity study and a 2-generation study (d-carvone, rats, by gavage) the DS concluded that there was no evidence of reproductive toxicity of carvone (both for effects on fertility and developmental toxicity). The DS proposed not to classify carvone for reproductive toxicity.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

In the 2-generation rat study oral dose levels of 0, 3, 10 and 30 mg/kg/d were tested (d-carvone). The high-dose was increased to 90 mg/kg/d when the F1 animals were 3-5 weeks old. The reported NOAEL for systemic toxicity is 30 mg/kg/d. No effects of carvone on reproductive parameters were observed. This applies both to reproductive parameters in both parental generations and to the F1 and F2 pups. RAC notes that the CLH dossier contains specific assessments of the relevant study parameters, however it does not consistently contain the original dose-response data (see table 22 in the CLH report). RAC had access to the study report and ran a plausibility check. The RAC considers the DS's toxicological assessment of the experimental data (2-generation study) as adequately convincing.

Tested dose levels in the oral teratogenicity study (GD 6-20) were higher than in the 2-generation study (d-carvone: 0, 20, 70 and 200 mg/kg/d). A small reduction of body weight gain was observed in the mid- and high-dose groups. In the highest dose group, there was one litter with ten dead foetuses. It was reported that the study author and reviewers considered this a chance finding. No other toxicologically relevant effects (neither maternal effects nor developmental toxicity) were reported (see table 23 of the CLH report). The RAC notes that the CLH dossier contains specific assessments of the relevant study parameters, however it does not consistently contain the original dose-response data. The RAC had access to the study report and ran a plausibility check. The RAC considers the DS's toxicological assessment of the experimental data (developmental toxicity study) as adequately convincing.

RAC agreed with the DS that there is no experimental evidence for fertility impairment or developmental toxicity of carvone. The RAC concludes that classification of carvone for reproductive toxicity (either fertility impairment or developmental toxicity) is not warranted.

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

There is no proposal for classification of the environmental hazards.

Degradation

The DS provided information on carvone's abiotic and biotic degradation. Carvone isomer ratio (d- and l-carvone) was not specified.

A hydrolysis study (OECD TG 111) was run at pH 4, 7 and 9. A decrease in concentration of <10% was observed after 5 days at 50°C. Therefore, the DS concluded that carvone is hydrolytically stable.

Information on biotic degradation in a screening test (ready biodegradation) was provided.
The screening test was performed with carvone at 1 and 3 mg/l in a closed bottle test (OECD TG 301D). After 28 days the degradation was 68 and 62%, at 1 and 3 mg/l respectively, with > 60% degradation achieved within a 14-days window.
The DS concluded that carvone is readily biodegradable.

Bioaccumulation
Carvone has a measured log kow of 2.4 (pH 4, 7 and 10 at 20°C, OECD TG 107).
No experimental studies on bioaccumulation of carvone in fish were submitted. The DS indicated that carvone has a low potential for bioaccumulation.

Aquatic toxicity
DS reported results of a short-term toxicity study with fish and an aquatic invertebrates test, other than an algae toxicity test. These tests were performed according to OECD test guidelines. Moreover, a toxicity test with *Lemna minor* was provided in accordance with ISO standard proposal (2000) and a draft OECD test guideline (1999).
No long-term toxicity tests are available.
A calculated 96h-LC_{50} of 67 mg/l, expressed as a nominal concentration, was reported as the results of the short-term toxicity to fish study, performed with a semi-static zebrafish toxicity test (OECD TG 203).
For the short-term toxicity to aquatic invertebrates, performed with a static *Daphnia* toxicity test (OECDTG 202), the DS reported a 48h-EC_{50} of 46 mg/l expressed as a nominal concentration.
For the algae toxicity test (OECD TG 201), an EC_{50} for growth rate of 41 mg/l was calculated. A 96h-NOEC for growth rate of 11 mg/l was estimated.
The toxicity test with *Lemna minor* provided a NOEC value of 10 mg/l.

Comments received during public consultation
No comments were submitted on environmental hazards. One MS expressed agreement with the DS on the proposal for no classification for the environmental hazards.

Assessment and comparison with the classification criteria
Degradation
A ready biodegradability test (OECD TG 301D) was performed with carvone (isomer ratio not specified). During 28 days the degradation was 62 to 68%, respectively. 60% degradation was reached within a 14-days window. Based on this result, the RAC considered carvone as readily biodegradable.

Bioaccumulation
The measured log kow value (log kow=2.4) submitted by the DS is considered reliable for classification purposes. No experimental studies into the bioaccumulation of carvone in fish were submitted; RAC agreed with DS that carvone has a low potential for bioaccumulation.

Aquatic toxicity
RAC agreed with the DS that carvone does not fulfil the criteria for classification under Directive 67/548/EEC or for classification under Regulation EC 1272/2008, because the provided L(E)C_{50} values for fish, daphnia, algae and aquatic plants are between 10 – 100 mg/l and the available NOEC values are > 1 mg/l.

However, the RAC emphasises that the reported experiments were performed at various concentrations, some at higher concentrations than the water solubility of carvone (29-79 mg/l). The results are based on the nominal and not the measured concentrations.

RAC also highlights that for the aquatic toxicity tests the ECHA guidance on information requirements and chemical safety assessment (chapter R.7.b: endpoint specific guidance) recommends that "exposure should be calculated in terms of geometric mean measured concentrations unless measured concentrations were within 20% of the nominal concentration, in which case the nominal concentrations may be used".

In assessing the experimental conditions reported by the DS, it is clear that measured (actual) concentrations were not determined at all levels, and for several samples the actual concentration was not satisfactorily maintained within 20% of the nominal concentrations. As a consequence, the results could not be based on nominal values.

In conclusion, the RAC is of the opinion (in agreement with the dossier submitter's proposal) that the information provided shows that carvone and carvone stereoisomers do not meet the criteria for classification for environmental toxicity.

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ANNEXES:

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

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and the RAC (excl. confidential information).