Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community 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/A1

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

Adopted

4 June 2013

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: **CARVONE**

EC Number: 202-759-5 (d/l mixture)
218-827-2 (d-carvone)
229-352-5 (l-carvone)

CAS Number: 99-49-0 (d/l mixture)
2244-16-8 (d-carvone)
6485-40-1 (l-carvone)

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Carvone
EC number:	202-759-5 (d/l mixture) 218-827-2 (d-carvone) 229-352-5 (l-carvone)
CAS number:	99-49-0 (d/l mixture) 2244-16-8 (d-carvone) 6485-40-1 (l-carvone)
Annex VI Index number:	-
Degree of purity:	The active substance shall have a minimum purity of 930g/kg carvone in the technical product with a d/l ratio of at least 100:1.
Impurities:	Confidential. No relevant impurities for the purpose of classification and labelling.

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	none	none
Current proposal for consideration by RAC	Skin irrit. Cat 2 Skin sens. Cat 1B	Skin irrit. R38 Skin sens. R43
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin irrit. Cat 2 Skin sens. Cat 1B	Skin irrit. R38 Skin sens. R43

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

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	Not classified	none	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	none	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	none	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	none	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	none	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	none	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	none	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	none	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	none	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	none	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	none	Not classified	conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	none	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	none	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Skin irrit. Cat 2	none	Not classified	
3.3.	Serious eye damage / eye	Not classified	none	Not classified	conclusive but not sufficient

	irritation				for classification
3.4.	Respiratory sensitisation	Not classified	none	Not classified	conclusive but not sufficient for classification
3.4.	Skin sensitisation	Skin sens. Cat 1B	none	Not classified	
3.5.	Germ cell mutagenicity	Not classified	none	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	none	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity – single exposure	Not classified	none	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	none	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	none	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Not classified	none	Not classified	conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	Not classified	none	Not classified	conclusive but not sufficient for classification

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

Labelling: Signal word: warning
Hazard statements: H315, H317
Precautionary statements: not relevant to Annex VI

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	Not classified	none	Not classified	conclusive but not sufficient for classification
Oxidising properties	Not classified	none	Not classified	conclusive but not sufficient for classification
Flammability	Not classified	none	Not classified	conclusive but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*	Not classified	none	Not classified	conclusive but not sufficient for classification
Thermal stability	Not classified	none	Not classified	conclusive but not sufficient for classification
Acute toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	Not classified	none	Not classified	conclusive but not sufficient for classification
Repeated dose toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Irritation / Corrosion	Skin irrit. R38	none	Not classified	
Sensitisation	Skin sens. R43	none	Not classified	
Carcinogenicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Mutagenicity – Genetic toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Not classified	none	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – development	Not classified	none	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	Not classified	none	Not classified	conclusive but not sufficient for classification
Environment	Not classified	none	Not classified	conclusive but not sufficient for classification

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: irritant
R-phrases: R38, R43
S-phrases: S(2-)24-37

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The classification and labelling has not been previously discussed at TC C&L.

2.2 Short summary of the scientific justification for the CLH proposal

There are currently no REACH registrations of carvone and its isomers (database accessed on 18-09-2012). The classification is based on the data as provided for the inclusion of carvone as a plant protection product in Annex I of Directive 91/414/EEC.

The substance should be classified as a skin irritant (category 2, H315) because desquamation persists through the end of the observation period in three tested animals.

Carvone scored positive in a GPMT test with 9/19 and 10/19 positive after challenge with 75 and 50% carvone (induction intradermal 5%), respectively. Therefore, this substance should be classified as a skin sensitiser (category 1B, H317).

2.3 Current harmonised classification and labelling

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

None

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

None

2.4 Current self-classification and labelling

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

Inventory notifications for CAS number 99-49-0 (accessed on 17-09-2012)

Classification		Labelling		Specific Concentration limits, M-Factors	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Pictograms Signal Word Code(s)		
Acute Tox 4 Skin Sens 1	H302 H317	H302 H317	GHS07 Wng		209
Skin Irrit 2 Skin Sens 1	H315 H317	H315 H317	GHS07 Wng		19
Flam Liq 1 Acute Tox 4	H224 H302	H224 H302	GHS07 GHS02		1

			Dgr		

Inventory notifications for CAS number 2244-16-8 (accessed on 17-09-2012)

Classification		Labelling		Specific Concentration limits, M-Factors	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Pictograms Signal Word Code(s)		
Acute Tox 4 Skin Sens 1	H302 H317	H302 H317	GHS07 Wng		914

Inventory notifications for CAS number 6485-40-1 (accessed on 17-09-2012)

Classification		Labelling		Specific Concentration limits, M-Factors	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Pictograms Signal Word Code(s)		
Acute Tox 4 Skin Sens 1	H302 H317	H302 H317	GHS07 Wng		1013
Acute Tox 4	H302	H302	Wng		34
Acute Tox 4	H302	H302	GHS07 Wng		27
Acute Tox 4 Skin Sens 1 Aquatic Chronic 3	H302 H317 H412	H302 H317 H412	GHS07 Wng		1

2.4.2 Current self-classification and labelling based on DSD criteria

No information on self-classification according to DSD criteria is available in the notifications.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No justification is required according to CLP Art 36(2) because carvone (d/l mixture with CAS number 99-49-0) is registered as a plant protection product according to Directive 2008/44/EC amending Directive 91/414/EEC. However, the substance identity (SID) used in Directive 2008/44/EC is not completely in agreement with ECHA's Substance Identity Guidance. A harmonized classification is proposed for the substance as defined in the Commission review report for the active substance carvone (SANCO/3920/2007-rev. final, 21 January 2008). Although d- and l-carvone cannot be considered to be toxicological identical substances, there is no information indicating that one of the isomers is clearly more toxic than the other.

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 (eco-)toxicological database consists of studies performed with d-carvone, l-carvone, or carvone with a (non-)specified isomer ratio.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

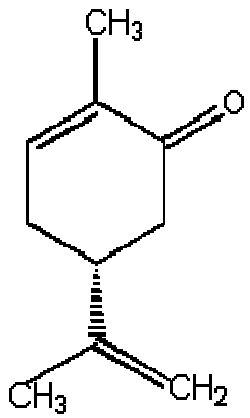
1.1 Name and other identifiers of the substance

Table 5: Substance identity

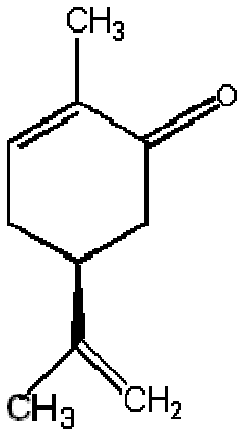
EC number:	202-759-5 (d/l mixture) 218-827-2 (d-carvone) 229-352-5 (l-carvone)
EC name:	2-methyl-5-(1-methylvinyl)cyclohex-2-en-1-one (d/l mixture) (*S*)-2-methyl-5-(1-methylvinyl)cyclohex-2-en-1-one (d-carvone) (*R*)-2-methyl-5-(1-methylvinyl)cyclohex-2-en-1-one for (l-carvone)
CAS number (EC inventory):	99-49-0 (d/l mixture) 2244-16-8 (d-carvone) 6485-40-1 (l-carvone)
CAS number:	99-49-0 (d/l mixture) 2244-16-8 (d-carvone) 6485-40-1 (l-carvone)
CAS name:	2-cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (d/l-mixture) 2-cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (5*S*)-, (d-isomer) 2-cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (5*R*)-, (l-isomer)
IUPAC name:	2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one (d/l-mixture) (5S)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one (d-carvone) (5*R*)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one (l-carvone)
ISO name	carvone
CLP Annex VI Index number:	none
Molecular formula:	$C_{10}H_{14}O$
Molecular weight range:	150.21

The substance identity as defined in Directive 2008/44/EC and in the review of carvone as a plant protection product are not completely in agreement with ECHA's Substance Identity Guidance document.

Structural formula:



l-carvone



d-carvone

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
l-carvone and d-carvone			The active substance shall have a minimum purity of 930 g/kg carvone in the technical product with a d/l ratio of at least 100:1 (defined in Directive 2008/44/EC)

Current Annex VI entry: none

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			No relevant impurities for the purpose of classification and labelling

Current Annex VI entry: none

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
				There are no additives.

1.2.1 Composition of test material

Carvone is a mixture of 2 stereoisomers: d- (or +)-carvone and l- (or -)-carvone. The (eco)toxicological database available consists of studies performed with d-carvone, l-carvone, or carvone with a nonspecified isomer ratio. Although d- and l-carvone cannot be considered to be toxicological identical compounds, there is no information indicating that one of the isomers is clearly more toxic than the other. For acute toxicity, irritation and sensitisation, carvone with a non-specified isomer ratio has been used (with the exception of acute inhalation toxicity, where a mixture with an isomer ratio of 4:1 is used). For repeated dose toxicity and mutagenesis, studies are performed either with d-carvone or with carvone with a non-specified isomer ratio. For carcinogenesis and reproduction toxicity, only studies with d-carvone were available. Therefore, the toxicity of the l- and d-isomer for these hazard properties cannot be compared based on the available data. In most studies using carvone with an unspecified isomer ratio, it is assumed that d-carvone was the main isomer. Therefore, all information available on d-, l-, and unspecified carvone is summarised below for evaluation.

1.3 Physico-chemical properties

Table 9: 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	Colourless to yellow liquid with a penetrating odour	DAR, 2006	
Melting/freezing point	-43°C	DAR, 2006	
Boiling point	233°C	DAR, 2006	
Relative density	0.96 kg/l	DAR, 2006	
Vapour pressure	1.9 Pa at 20 °C	DAR, 2006	
Surface tension	57.2 mN/m for a 90 % saturated concentration in water at 20 °C	DAR, 2006	
Water solubility	27 to 79 mg/l at 20 °C (no pH dependency)	DAR, 2006	
Partition coefficient n-octanol/water	2.4 (at pH 4, 7 and 10) at 20 °C	DAR, 2006	
Flash point	98 °C	DAR, 2006	
Flammability	flash point: 98^0C	DAR, 2000	
Explosive properties	Not explosive	DAR, 2006	
Self-ignition temperature	295^0C	DAR, 2000	
Oxidising properties	Not oxidising	DAR, 2000	
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	Carvone has no groups which will dissociate in a relevant pH range (2-10)	DAR, 2006	
Viscosity	No data		
Volatility; Henry's law constant	3.6-10.6 Pa m^3 mol^{-1} (as a range because water solubility was given as a range)	DAR, 2000	calculated

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this type of report.

2.2 Identified uses

Carvone (d/l) is used in several food stuffs as a flavouring agent. L-carvone is added to chewing gum and d-carvone has been added to food stuff such as biscuits, candies, bread, and meat. Further, d-carvone has been found in (non-)alcoholic beverages. In addition, carvone (d/l) is used in nonfood products. Carvone (d/l) is used in personal care products as a flavour and fragrance agent in toothpaste, mouthwash, soap, and perfume. D-carvone also proved a successful plant growth regulator and pesticide on potato crops, e.g. to prevent or regulate the sprouting of dormant ware and dormant starch potatoes (Wolterink et al., 2009).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1 Physico-chemical properties

3.1.1 Summary and discussion of physico-chemical properties

Carvone has a flash point of 98^0C, is not explosive and not oxidising.

3.1.2 Comparison with criteria

A liquid should be classified as flammable when the flash point is at or below 60°C. Carvone does not meet this criterion.

3.1.3 Conclusions on classification and labelling

Carvone does not need to be classified for physico-chemical perperties in both the CLP Regulation and the DSD.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

For the present evaluation an ADME study in the rat was not available. Four studies on the kinetics of carvone in volunteers and an in vitro metabolism study using human and rat liver microsomes were available. Incubation of l- and d-carvone with rat liver microsomes yielded l- and d-carveol. Only l-carveol was glucuronidized.

4.1.2 Human information

In male volunteers an oral administration of 100 mg/subject resulted in peak blood concentrations of 15 ng/ml 1.3 h after administration. The elimination of carvone from the blood appears to be rapid, with a calculated half-life in blood of 2.5 h. In a second study in male volunteers, carvone rapidly penetrated the skin. 30 Min following a dermal application of l- and d-carvone in volunteers, peak concentrations in blood were observed. Peak levels for l-carvone were 3.5 times higher than for d-carvone. Calculated half-lives in blood were 33.5 and 37.5 min for l- and d-carvone respectively. The level of excretion of l- and d-carvone in urine was low. After 24 h 1.2

and 1.3% of respectively l- and d-carvone were excreted in urine. Low quantities (<0.25% of dose) of the l-carvone metabolites 4R,6S-(-)-carveol and 4R,6S-(-)-carveol glucuronide were detected.

In two volunteer studies the metabolism of l- and d- carvone was analysed qualitatively and semi-quantitatively. In one study, major metabolites were dihydrocarvonic acid, carvonic acid and uroterpenolone. Minor metabolites were carveol and dihydrocarveol. A second volunteer study indicated that through one metabolic pathway carvone was oxidised and subsequently hydrolysed to yield uroterpenolone. Alternatively, carvone could be oxidised to carvonic acid or dihydrocarvonic acid. Incubation of l- and d-carvone with human liver microsomes yielded l- and d-carveol. Only l-carveol was glucuronidized.

4.1.3 Summary and discussion on toxicokinetics

Based on the data present in the draft assessment report and the addendum no firm conclusion on the similarities and differences in metabolism of carvone between humans and animals can be drawn. The data provide no information on the percentage absorption after oral, dermal or inhalatory exposure, the distribution of carvone or the rate and routes of elimination from the body.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
OECD TG 401	LD50 > 2000 mg/kg bw	rats	DAR, 2000
OECD TG 402	LD50 > 4000 mg/kg bw	rats	DAR, 2000
OECD TG 403	LC50 > 5.66 g/m3	rats	DAR, 2000

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

One study was performed in accordance with OECD 401. Carvone with a nonspecified isomer ratio was used in this study at a single dose of 2000 mg/kg bw. There was no mortality. Clinical signs observed after acute oral administration included hunched posture and lethargy, and in 2 animals occasional body tremor was noted. No abnormalities were observed at necropsy. The oral LD50 of carvone in rats was >2000 mg/kg bw.

NTP (1990) mentions another acute oral toxicity study, resulting in an LD50 of 1640 mg/kg bw in rats and 766 mg/kg bw in guinea pigs (Jenner, 1964). However, the purity of carvone used in this study is unknown. In addition, the details of this study are very limited. The study is therefore considered as unreliable.

4.2.1.2 Acute toxicity: inhalation

One study was performed in accordance with OECD 403. Carvone with a *d/l* isomer ratio of minimally 4:1 was used in this study at a single dose of 5.66 g/m^3. One female died the day after the exposure. During exposure a decreased breathing frequency, and less frequently, post-inspiratory apnoea and superficial breathing were observed. After exposure and increased breathing frequency, post-inspiratory apnoea and dyspnoea were seen. Clinical signs during exposure were restlessness and stress and incoordination and tremors. A dirty and wet fur was observed 24-48h after treatment.

Alopecia was observed in a few rats at days 7-13. Body weight gain was impaired in most rats during the first week after treatment. Normal body weight gain was observed in the second week, except for two females that showed only marginal body weight gain.
Pathology revealed no abnormalities, except in the female that died the day after exposure: dark foamy lungs, light coloured liver and air-filled stomach and intestines were observed. The respiratory LC50 of carvone in rats was >5.66 g/m^3.

4.2.1.3 Acute toxicity: dermal

One study was performed in accordance with OECD 402. Carvone with a nonspecified isomer ratio was used in this study at a single dose of 4000 mg/kg bw. No vehicle was used. There was no mortality. After acute dermal exposure no systemic or skin effects were seen. The dermal LD50 of carvone in rats was >4000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

The oral LD50 in rat was >2000 mg/kg bw *(ratio d/l unspecified)*, the dermal LD50 in rat was > 4000 mg/kg bw *(ratio d/l unspecified)*, and the inhalation LD50 in rat was > 5.66 g/m3 (*d/l* isomer ratio of minimally 4:1)). No mortality was observed after oral and dermal exposure; after inhalation one female died the day after exposure.
Clinical signs observed after acute oral administration included hunched posture and lethargy, and in 2 animals occasional body tremor was noted. No abnormalities were observed at necropsy. After acute dermal exposure no systemic or skin effects were seen. After inhalation exposure respiratory effects were noted, alopecia was observed, and body weight gain was impaired.

4.2.4 Comparison with criteria

According to the criteria of the DSD, substances should not be classified when: oral LD50>2000 mg/kg bw; dermal LD50 > 2000 mg/kg bw and inhalation LC50> 5 mg/l (dusts and mists). Carvone does not meet the DSD criteria.

According to the criteria of the CLP Regulation, substances should not be classified when: oral LD50>2000 mg/kg bw; dermal LD50 > 2000 mg/kg bw and inhalation LC50> 5 mg/l (dusts and mists). Carvone does not meet the CLP criteria.

4.2.5 Conclusions on classification and labelling

Carvone does not need to be classified for acute oral, dermal and inhalation toxicity in both the CLP Regulation and the DSD.

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).

Additional key elements

Key elements

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.

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

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

Clinical signs observed after acute oral administration (2000 mg/kg bw) included hunched posture and lethargy, and in 2 animals occasional body tremor was noted. No abnormalities were observed at necropsy. After acute dermal exposure (2000 mg/kg bw) no systemic or skin effects were seen. After inhalation exposure (5.66 g/m3) respiratory effects were noted, alopecia was observed, and body weight gain was impaired.

4.3.2 Comparison with criteria

According to the criteria of the CLP Regulation for single dose exposure, substances should not be classified when single dose at: oral >2000 mg/kg bw; dermal > 2000 mg/kg bw and inhalation > 5 mg/l (dusts and mists). Carvone does not meet these CLP criteria. In addition, substances can be classified for STOT-SE based on respiratory tract irritation. Although respiratory effects as altered breathing frequency, post-inspiratory apnoea and superficial breathing were observed in some animals, these effects do not necessarily indicate irritation. Since no direct indications of irritation were observed (as redness or oedema), carvone does not meet the criteria for classification based on respiratory tract irritation.

4.3.3 Conclusions on classification and labelling

No classification for acute toxicity is required for carvone.

4.4 Irritation

4.4.1 Skin irritation

Table 11: Summary table of the skin irritation study

Scores observed after	30-60 minutes	24 hours	48 hours	72 hours	7 days
Erythema	1,1,1	1,2,1	1,2D,0	1D,2D,0	0D,0D,0D
Edema	1,2,1	1,2,0	0,1,0	0,1,0	0,0,0

D=desquamation

4.4.1.1 Non-human information

One skin irritation study was performed in accordance with OECD guideline 404. Carvone (isomers not specified) was mildly irritant to rabbit skin (Table 11). Desquamation persisted through the end of the observation period. The extent of desquamation was not reported.

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

Carvone is mildly irritating to the skin. Desquamation persists through the end of the observation period. The extent of desquamation was not reported.

4.4.1.4 Comparison with criteria

The mean value of the scores for either erythema and eschar formation or oedema calculated is less than 2, the cut off value for classification according to DSD. However, since desquamation persists in more than 2 animals through the end of the observation period, carvone does fulfil the DSD criteria for classification as Xi and R38.

The mean value of the scores for either erythema and eschar formation or oedema per animal is too low to fulfil the classification criteria according to the CLP regulation (<2.3). However, because desquamation persists through the end of the observation period in 3 tested animals, carvone fulfils the criteria (number 2 of table 3.2.2) for classification as a skin irritant (category 2, H315) of the CLP Regulation.

4.4.1.5 Conclusions on classification and labelling

Classification is required for a substance with persistent irritation to the skin. Carvone is classified as a skin irritant with Xi and R38 according to the DSD and as category 2 irritant (H315) according to the CLP Regulation because desquamation persists through the end of the observation period.

4.4.2 Eye irritation

Table 12: Summary table of the eye irritation study

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
Conjunctiva redness	1,1,1	2,1,1	1,0,0	0,0,0
Conjunctiva chemosis	1,1,1	1,0,0	0,0,0	0,0,0
Conjunctiva 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).

4.4.2.1 Non-human information

One eye irritation study was performed in accordance with OECD guideline 405. Carvone (isomers not specified) was found to be mildly irritating to the rabbit eye (Table 12).

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation

Carvone was found to be mildly irritating to the rabbit eye.

4.4.2.4 Comparison with criteria

Carvone was found to be mildly irritating to the rabbit eye. However, the mean value of the scores for corneal opacity, iris lesions, conjunctival redness and conjunctival oedema for each animal are below the cut-of values for classification according to the CLP Regulation (1, 1, 2 and 2, respectively in 2 out of 3 animals).

Carvone was found to be mildly irritating to the rabbit eye. However, the mean value of the scores for corneal opacity, iris lesions, conjunctival redness and conjunctival oedema are below the cut-of values for classification according to the DSD Regulation (2, 1, 2.5 and 2, respectively).

4.4.2.5 Conclusions on classification and labelling

Carvone does not need to be classified for eye irritation in both the CLP Regulation and the DSD.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data available. The skin and eye irritation study (4.4.1) shows some irritation potential. In the acute inhalation study, during and shortly after exposure respiratory changes were observed. During exposure a decreased breathing frequency, and less frequently, post-inspiratory apnoea and superficial breathing were observed. After exposure an increased breathing frequency, post-inspiratory apnoea and dyspnoea were seen. Pathology revealed no abnormalities, except in the female that died the day after exposure: dark foamy lungs, light coloured liver and air-filled stomach and intestines were observed. The respiratory LC50 of carvone in rats was >5.66 g/m3.

4.4.3.2 Human information

No data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

Although respiratory effects as altered breathing frequency, post-inspiratory apnoea and superficial breathing were observed in some animals in the acute inhalation study, these effects do not necessarily indicate irritation. Since no direct indications of irritation were observed (as redness or oedema), carvone does not meet the criteria for classification based on respiratory tract irritation.

4.4.3.5 Conclusions on classification and labelling

Carvone does not need to be classified for respiratory tract irritation in the DSD regulation.

4.5 Corrosivity

4.5.1 Non-human information

No data available.

4.5.2 Human information

No data available.

4.5.3 Summary and discussion of corrosivity

The skin irritation study (4.4.1) shows no signs of corrosion.

4.5.4 Comparison with criteria

The skin irritation study (4.4.1) shows no need for classification for corrosion in both the CLP Regulation and the DSD.

4.5.5 Conclusions on classification and labelling

Carvone does not need to be classified for corrosivity in both the CLP Regulation and the DSD.

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.

Additional key elements

Key elements

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 area of opacity	d, 0,0 4,0,0	1,0,0 2,0,0	1,0,0 1,0,0	0,0,0 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.

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information

One skin sensitisation GPMT study was performed in accordance with OECD guideline 406. After dermal challenge with topical 75% carvone (isomers not specified, induction intradermal 5%, topical undiluted) 9/19 animals responded with slight to moderate erythema at 24h with 5 animals showing a reaction extending beyond the test site. At 48h, only 1 animal showed erythema but in 3 animals desquamation was observed. After challenge with topical 50% carvone, 10/19 animals showed erythema at 24h of which 4 animals showed an extended reaction. At 48h, 1 animal showed erythema but two showed desquamation. In control animals, no erythema reactions were observed at all.

4.6.1.2 Human information

Several case studies are available in which patch tests for (l-)carvone were positive (Worm, 1998; Corazza, 2002; Quertermous, 2010). In addition, patch tests for l-carvone were positive in 15 out of 541 patients with contact allergy (Paulsen, 1993). All cases had used spearmint toothpaste, spearmint chewing gum or shampoo with a mint scent

4.6.1.3 Summary and discussion of skin sensitisation

Carvone scored positive in a GPMT test (9/19 and 10/19 positive after challenge with topical 75 and 50% carvone, respectively *(ratio d/l unspecified).*

4.6.1.4 Comparison with criteria

In the DSD, a substance should be classified with Xi and R43 according to EU labelling criteria, when more than 30% of the animals showed a positive response. This criterion is fulfilled.

In the CLP Regulation, a substance should be classified as a skin sensitiser (category 1, H317) when a positive response in a GPMT test (in >30% of the animals) is observed. This criterion is fulfilled. Subcategory 1B is required when ≥30% responses at > 1% intradermal induction dose or when the substance shows a low to moderate frequency of sensitisation in humans.

4.6.1.5 Conclusions on classification and labelling

Carvone should be classified with Xi and R43 according to the DSD and Skin Sens Cat1B: H317 according to the CLP Regulation, respectively.

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. 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.

4.6.2 Respiratory sensitisation

No data available.

4.6.2.1 Non-human information

No data available.

4.6.2.2 Human information

No data available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data available. There is no need for classification for respiratory sensitisation.

4.6.2.4 Comparison with criteria

No data available.

4.6.2.5 Conclusions on classification and labelling

There is no need for classification for respiratory sensitisation, based on absence of data.

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.

4.7 Repeated dose toxicity

The results of the relevant subacute and (sub)chronic toxicity studies are summarised in the following table.

Table 13: Summary table of relevant repeated dose toxicity studies

Duration	Species	Dose (mg/kg bw/day)	Results	Remarks
14 days	rats	0, 50, 200, 1000	50 mg/kg bw/day	NOAEL
16 days	mice	0, 150, 328, 723, 1590, 3000		NOAEL cannot be established
90 days	rats	0, 5, 30, 180	5 mg/kg bw/day	NOAEL
13-weeks	mice	0, 93, 187, 375, 750, 1500	375 mg/kg bw/day	NOAEL
28-weeks	rats	0, 50, 125, 500		NOAEL cannot be established
2 years	mice	0, 375, 750	375 mg/kg bw/day	LOAEL

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

In a subacute study with rats (14 days, carvone, *ratio d/l unspecified)* 100% mortality was observed at levels of 1000 mg/kg bw/day while clinical signs were dose-related increased at 200 and 1000 mg/kg bw/day. At 200 mg/kg bw, slights effects were noted on haematological and biochemical parameters and absolute and relative kidney weight of males was significantly increased. Macroscopy revealed effects on the forestomach only in dead animals. The NOAEL in this study was 50 mg/kg bw/day.

In a subacute study with mice (16 days, d-carvone) 100% mortality was observed at 1590 mg/kg bw/day and above while clinical signs were dose-related increased at 723 mg/kg bw/day and above. Relative liver weights were increased and thymus weights decreased in all dose groups. In addition, food intake, haematological and biochemical analysis were not performed. Therefore, a NOAEL cannot be established in this study.

In a well performed 90-day study (carvone, *ratio d/l unspecified*) with rats clinical signs and slight deceases in body weight gain were observed at the highest dose (180 mg/kg bw/day). At 30 mg/kg bw/day and above various haematological and biochemical effects were observed. Absolute and relative liver and kidney weights were dose-relatedly increased (absolute liver weight <20%, absolute kidney weight up to 43%) while thymus weights were decreased (<20%). Macroscopy revealed enlarged kidneys at 180 mg/kg bw/day and histopathological analysis showed tubular necrosis at 30 mg/kg bw/day and above. The mechanism of kidney toxicity was further addressed. Microscopical re-evaluation of the kidney slides revealed in males of the 30 and 180 mg/kg bw/day groups slight to severe tubular changes, characterised by severe proteinaceous (hyalin) droplets within the proximal tubular cells, accompanied by epithelial cell necrosis and the occurrence of granular casts in the outer medulla, and proteinaceous casts and regenerating tubules. In addition, the staining with antibody against α_{2u}-globulin showed highly positive staining in the kidney slides of males of the high dose group.It is concluded that the renal histopathological changes in the kidney of male rats treated with carvone at doses of 30 and 180 mg/kg bw/day are the result of accumulation of α_{2u}-globulin in the proximal tubular cells. As this protein is not present in higher mammals including man, these α_{2u}-globulin-related effects can be considered not relevant for exposure risk assessment of carvone in man. The NOAEL in this study is 5 mg/kg bw/day.

Table 14: Summary table of the repeated dose toxicity study

Dose groups mg/kg bw/day	**0**		**5**		**30**		**180**		**dose related**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No mortality occurred								
Clinical signs									
-alopecia	4/10	5/10	1/10	1/10	0/10	0/10	4/10	4/10	
-salivation	0/10	1/10	1/10	3/10	2/10	2/10	10/10	10/10	
-rales	1/10	1/10	0/10	0/10	1/10	1/10	2/10	2/10	
Body Weight (gain) [a]							(d)	(d)	
Food Consumption [b]							i	i	
Ophthalmoscopy	No toxicological relevant effects								
Haematology									
-RBC							d	i	
-MCV							i		
-PT							ds		
-PTT						is		is	dr
Blood Biochemistry									
-albumin						ds		ds	
-ALAT							d	d	
-ASAT					d		ds	d	
-Bilirubin							ds		
-Cholesterol							i		
-Triglycerids					i		i		
-Cl					ds		ds		
-inorg. P					ds		ds		
-Ca				ds		ds		ds	
Organ Weights abs									
-liver	15.43	8.01	14.61	8.34	15.16	8.92	16.20	9.28 is	dr
-kidneys	3.02	1.63	3.07	1.69	3.42	1.85 is	4.33 is	1.95 is	dr
-thymus		0.35		0.33		0.28 ds		0.30	
Organ Weights rel									
-liver	2.95	2.75	2.83	2.78	2.94	3.09 is	3.24 is	3.23 is	dr
-kidneys	0.58	0.56	0.60	0.57	0.66 is	0.64 is	0.87 is	0.68 is	dr
-thymus		0.12		0.11		0.10 ds		0.10 d	
-ovaries								i	
Macroscopy									
-Kidney enlarged [c]	0/10	0/10	0/10	0/10	0/10	0/10	8/10	0/10	
Microscopy									
-Kidney									
basophilic tubules	5/10	0/10	6/10	0/10	1/10	1/10	0/10	1/10	
tubular necrosis	0/10	0/10	0/10	0/10	8/10	0/10	10/10	1/10	dr

m/f = male/female, i/d = increased/decreased, is/ds = increased/decreased significantly, np = not performed, a/r = absolute/relative, dr = dose-related. To assess significance Dunnett test, Steel test and the exact Fisher test (opthalmoscopic data) were used.

a) Body weight gain of high dose animals was slightly decreased in week 4-6.

b) Overall, the food intake of high dose animals was higher: mean values over the whole treatment period for males (and females) were 62 (70), 63 (72), 63 (70), and 71 (77) g/kg bw/day for the 0, 5, 30, and 180 mg/kg bw/day dose groups respectively.

c) Besides enlargement, kidneys of the high dose group were discoloured, pale, and granular.

In a 13-week gavage study (d-carvone) with mice mortality and clinical signs were observed at 1500 mg/kg bw/day. At 750 mg/kg bw/day, relative liver weight was significantly increased. The NOAEL in this study is 375 mg/kg bw/day, but food intake, haematological and biochemical analysis were not performed.
It has been demonstrated that oral treatment of rats with 2500 mg/kg food for 1 year (equivalent to 125 mg/kg bw/day) and 1000 mg/kg food for 28 weeks (equivalent to 50 mg/kg bw/day) showed no effects. At 10000 mg/kg food for 16 weeks (equivalent to 500 mg/kg bw/day) growth retardation and testical atrophy were observed. However, since these studies showed major shortcomings compared to current guidelines these results can be used as indicative only.

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

No data available.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

In a 90 days gavage study in rats it was concluded that treatment with d-carvone induced enlarged kidneys and tubular necrosis in males, and increases in partial thromboplastine time (PTT), liver and kidney weights, and significant decreases in serum albumin, Ca and thymus weight in female rats at doses of 30 or 180 mg/kg bw/day. It is concluded that the renal histopathological changes in the kidney of male rats treated with carvone at doses of 30 or 180 mg/kg bw/day are the result of accumulation of α_{2u}-globulin in the proximal tubular cells. As this protein is not present in higher mammals including man, these α_{2u}-globulin-related effects can be considered not relevant for exposure risk assessment of carvone in man. However, since in the females of the 30 mg/kg bw/day group the absolute and relative increases in liver and kidney weight and the decrease in absolute and relative thymus weight were larger than 10 % (11-20%), the effects at 30 mg/kg bw/day were considered toxicologically relevant. Nevertheless, since no histopathological changes were observed in the liver and thymus, the changes in the weight of these organs are not considered to be serious damage.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

Increased liver and kidney weights, decreased thymus weight, and macroscopically and microscopically changes in the kidneys especially in males were observed at oral doses of 30 or 180 mg/kg bw/day. The renal histopathological changes in the kidney of male rats treated with carvone at doses of 30 or 180 mg/kg bw/day are the result of accumulation of α_{2u}-globulin in the proximal tubular cells, which is not considered relevant for man. No histopathological changes in liver and thymus were described. Significant decreases in serum calcium levels were observed in all treated female groups. However, since no changes in calcium were observed in males and, moreover, a dose-relationship was lacking, this effect is considered not to be toxicological relevant. In addition, another study with oral treatment of rats at doses of 50 and 125 mg/kg bw/day for 28 weeks showed no effects. Taken together, these studies suggest that carvone-induced effects are not severe at doses ≤ 100. No dermal and inhalation data are available for carvone.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

The cut-off value for R48/22 is 50 mg/kg bw/day in the DSD (in a 90 day repeated dose study). Significant effects on weights of kidney, liver, and thymus as well as serum parameters have been observed at doses of 30 mg/kg bw/day in a 90 day repeated dose study. Microscopical changes, however, were only observed in kidney and are considered not relevant for man. Since no histopathological changes were observed in the liver and thymus, the changes in the weight of these organs are also not considered to be serious damage. Some changes in serum parameters are considered not exposure related because a dose-relationship is lacking. Thus the effects observed at the dose of 30 mg/kg bw/day in the 90 day repeated dose study are considered not severe enough for R48 classification. This conclusion is supported by another study in which no effects were observed at doses of 50 and 125 mg/kg bw/day for 28 weeks of treatment.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Carvone does not need to be classified for repeated dose toxicity according to DSD.

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 1y	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).

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

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

Please see 4.7.1.8.

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

The cut-off values for STOT RE2 are $10 < C \leq 100$ mg/kg bw/day in the CLP Regulation (in a 90 day repeated dose study). Although significant effects on kidney, liver, thymus and serum parameters have been observed at doses of 30 and 180 mg/kg bw/day, these effects do not indicate organ dysfunction and therefore are considered not severe enough for STOT RE2 classification (see 4.7.1.9).

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

Carvone does not need to be classified for STOT RE according to CLP.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 15: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
OECD TG 471	negative	Ames test	DAR, 2000
OECD TG 471	negative	Ames test	DAR, 2000
OECD TG 476	equivocal	Gene mutation in mouse lymphoma cells	DAR, 2000
OECD TG 473	Positive (-S9) Negative (+S9)	Chromosome aberrations in human lymphocytes	DAR, 2000
OECD TG 473 like protocol	equivocal	Chromosome aberrations in CHO cells	DAR, 2000
OECD TG 479	positive	Sister Chromatid Exchanges in CHO cells	DAR, 2000
	negative	in vivo micronucleus test	DAR, 2000
OECD TG 468	negative	in vivo UDS assay in the liver	DAR, 2005

4.9.1 Non-human information

4.9.1.1 In vitro data

In an Ames test, carvone (isomer ratio not specified) was tested in triplicate in two independent experiments. Cytotoxicity was monitored in a preliminary test showing that the number of colonies was dose-dependently reduced without S9-mix at 333 and 1000 µg/plate with no colony growth at 3330 and 5000 µg/plate (-S9). In the presence of S9-mix colony reductions were observed at 1000 and 3330 µg/plate while at 5000 µg/plate no colony growth was observed.
In the absence of S9, in the first experiment a slight increase in the number of revertant colonies was observed for TA1535 at the highest dose whereas in the second experiment a slight increase was observed for TA1537 at the two highest dosages. However, both increases were marginal and were not observed in the independent duplo experiment (see Table 16). Under the experimental conditions used, carvone is considered to be non-mutagenic in Salmonella typhimurium.

Table 16: Mean number of revertant (His+) colonies/3 replicate plates (± S.D.) with different strains of S. typhimurium

Dose	TA98		TA100		TA1535		TA1537	
(ug/plate)	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Exp. 1:								
0[a]	12± 3	25± 3	134± 28	143± 6	9±2	10± 2	12± 0	5±1
10	17± 5		120;133[b]		5± 3		6± 3	
33	18± 4	25 ± 4	118± 12	148± 11	7± 1	10± 2	9± 4	5 ±2
100	13± 6	19± 2	134± 17	143± 12	6± 3	5± 3	4± 2	5± 3

333	13±5	24± 3	118± 12	159± 20	11± 5	4± 1	6± 1	9±4
1000	14± 5	24±6	127± 21	125± 11	16±1	4± 1	6± 1	7± 3
3330		15±5		5±6[d]		3±2[c]		0±1[e]
Pos. contr.	130±20	557± 67	944± 21	912± 61	236± 21	114± 15	604±133	241± 12
Exp. 2:						f		f
0[a]	14± 3	26± 5	134± 12	159± 11	8± 3	7± 0	8± 6	7± 1
10	14± 6		140 ± 1		11± 3		7± 4	
33	15± 2	21± 4	141± 13	151± 18	8± 2	6± 2	8±2	8±2
100	13± 7	15± 5	149± 5	139± 10	11± 2	8± 2	6± 3	5±1
333	16± 2	26± 4	151± 19	162± 6	9± 4	7± 2	12± 3	7± 4
1000	14± 2	25±3	109± 5	138± 15	9± 3	3± 1	14± 3	4± 3
3330		8±2[c]		46± 12[d]		2±1[c]		1±0[d]
Pos. contr.	162± 17	784± 45	1125±58	990±43	337± 23	318± 37	451± 44	232± 35

a) 0.1 ml DMSO
b) One plate infected with other bacteria
c) Bacterial backround lawn slightly reduced
d) Bacterial backround lawn moderately reduced
e) Bacterial backround lawn extremely reduced
f) Assay with S9-mix was performed in an independent experiment

In a second Ames test, carvone (isomer ratio not specified) was tested in triplicate in two independent experiments. The highest dosage was limited by toxicity (not defined) or solubility. None of the trials (either without S9 or in the presence of S9 (either from rat or syrian hamster liver) did show any increase in the number of revertant colonies. Under the experimental conditions used, carvone is considered to be non-mutagenic in Salmonella typhimurium (see table 17).

Table 17: Mean number of revertant (His+) colonies/3 replicate plates (± S.D.) with different strains of S. typhimuriuma

Dose	**TA98**		**TA100**		**TA1535**		**TA1537**	
(ug/plate)	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
Exp. 1:								
0	16±2.9	25± 2.6/ 27±3	97± 2	156±13/ 145±13	5±1.5	11± 2/8±1.2	4± 0.9	5±0.6/ 6±1

3.3	12±1	29±1/ 36±1	108±13	121±4/ 144±6	6± 1	7±2/ 5±0.3	3± 0.3	4±0.3/ 3±0.6
10	18± 3	25 ± 1/ 27±1	91± 1.5	137±6.5/ 131±9	5± 1	10± 1.5/ 3±0.3	2± 0.3	4 ±1/ 5±0
33	15± 2	26± 1/ 29±1	105±3	123± 4/ 143±4	5±1	6±1/3±1	2±1	5±1/ 8±0.3
100	17±2	25±1/ 21±1	95± 5	115±7/ 121±6	1± 0.6	4± 1/ 7±0.3	toxic	6±2/ 3±0.3
333	15±2	27±3/ 22±1	71±4	94±6/ 104±10	2±1	10±1/ 7± 0.3	toxic	1±0.3/ 4±0.3
Pos. Contr.	305±47	1741± 264/ 1815±76	492±75	1631±12 12/ 2162 ±100	260±13	109± 8/ 192±13	543±68	65±2/ 125±16
Exp. 2:								
0	14±2	21± 4/ 25±1	89± 5	133± 12/ 6±2	4± 1.5	8±3/ 6±1.5	3± 1	6± 1/ 6±1
3.3	16±3	20±3/ 23±5	84 ± 8	94±14/ 119±10	3± 1	5±0.3/ 5±1	3± 1	5±1/ 5±1
10	15±0.3	15± 3/ 23±1	75± 4	105± 7/ 113±10	3± 1	5± 1/4±1	2±0	8±1/ 5±1
33	11±1	22±1/ 25±3	89±4	108±2/ 107±7	3±0.3	4±1.5/ 3±1	3±1	7±2/2±1
100	13±1	22±2/ 23±2	73± 7	100±5/ 104±3	2±1	3±1/5±1	3± 1	8±3/ 4±1
333	10± 2	22±2/ 17±4	48±9	65±11/ 78±8	1±1	3±2/ 4±1	3±1	6±1/ 6±1
Pos. Contr.	359±21	973±201 1969±57	224±7	3091± 157/ 3096± 178	122±11	76±13/ 41±6	1041± 154	211±24/ 261±12

a) The results from the experiment with Aroclor 1254-induced S9 from male rat liver and male hamster liver are presented in one colmn (rat\ hamster)

In a gene mutation test with mouse lymphoma cells (carvone, ratio d/l unspecified), an equivocal response was observed because slight increases in mutation frequency were noted only at cytotoxic concentrations (see Table 18). Cloning efficiency in the main test directly after treatment was dose-dependently decreased in the presence of S9-mix (up to 25% survival at 333 µg/ml) but only decreased to 43% of control at the highest dose in the absence of S9. After 3 days of expression no effect of treatment was observed on the cloning efficiency. The mutation frequency was slightly increased at the highest dosage in all tests (less than a factor 2 (-S9) or about a factor 2 (+S9). The highest dose with metabolic activation was 333 µg/ml and was associated with emulsification.

Table 18: Cytotoxic and mutagenic response of carvone in the mouse lymphoma L5178Y test system

Dose	C.E. at day 0 (% of control)		C.E. at day 3 (absolute %)		mean no. Of mutants per plate		mutation frequency x 10^5	
(ug/ml)	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Exp. 1:								
0	100	100	69	68	0.8	0.7	0.8	0.7
33	101		67		0.7		0.7	
100	102		62		0.8		0.9	
133	102	87	67	61	0.4	0.7	0.4	0.8
178	43	90	65	64	1.1	0.5	1.1	0.5
237		68		59		0.6		0.7
333		25		70		1.8		1.7
0.5mM DMN		50		49		6.0		8.2
2 mM EMS	91		71		8.8		8.3	
Exp. 2:								
0	100	100	72	77	1.9	1.5	1.8	1.3
100	83	91	76	85	1.2	2.1	1.1	1.7
133	92		80		1.7		1.4	
178	66	67	68	68	0.7	1.6	0.7	1.5
237	112	73	72	72	3.2	1.6	3.0	1.5
333		73		64		3.0		3.1
0.5mM DMN		51		44		6.8		10.2
2 mM EMS	125		73		8.4		7.7	

Solvent control=DMSO

C.E. = Cloning Efficiency

EMS = Ethylmethanesulphonate

DMN = Dimethylnitrosamine

In an in vitro chromosome aberration test with human lymphocytes (carvone, ratio d/l unspecified), an increase in aberrations was found in the absence of metabolic activation but not in its presence. In the main test, MI was decreased below 50% only at 24h at 178 µg/ml (-S9). In the presence of S9, MI was maximally reduced to 55% of control at 333 µg/ml. In the absence of metabolic activation, carvone induced a significant increase in the number of cells with chromosome aberrations (excluding gaps) at 178 µg/ml in both experiments at 24h. At 100 µg/ml only a small but evident increase in aberrant cells was observed. The main type of aberration observed were

chromosomal breaks. At 48h, a small increase in aberrant cells was observed for 75 µg/ml but not for 100 µg/ml. In the presence of metabolic activation, no evident increase of chromosome aberrations was observed but two cases of polyploidy were reported at the highest dose.
In another chromosome aberration test with Chinese Hamster Ovary (CHO) cells (d-carvone), a significant increase in the percentage of aberrant cells was observed at 12.5 and 25 µg/ml in the first trial without metabolic activation. In the second trial a significant increase was observed only at 31.3 µg/ml but not at other (higher) concentrations. In the presence of metabolic activation, an increase in the percentage of aberrant cells was observed at the highest dose in both trials. However, in trial 1 the highest dose was 250 µg/ml whereas it was 400 µg/ml in the second trial.

A test for Sister Chromatid Exchanges (SCE) in CHO cells (d-carvone) was considered positive since in all trials a significant increase (>20%) in the number of SCE was observed.

4.9.1.2 In vivo data

An in vivo micronucleus test (carvone, ratio d/l unspecified) in mice using intraperitoneal injection of 1000 mg/kg bw was performed. At this dose all animals showed lethargy, no reaction to stimuli, and slow breathing. No mortality occurred. The PCE/NCE ratio was slightly, but not significantly, decreased at 48h in both sexes. No increase in the frequency of micronucleated cells was observed at any time-point. It was concluded that carvone is non-genotoxic (See table 19).

Table 19: Mean number of micronuclei per 1000 polychromatic erythrocytes and ratio of polychromatic/normochromatic erythrocytes

Group	Treatment	Dose (mg/kg bw)	Sampling time (hours)	No. of micronuclei per 1000 polychromatic erythrocytes (mean ± S.D) [1]	Ratio of polychromatic/ normochromatic erythroytes
Males					
A	Vehicle		24	0.4 ± 0.5	0.98 ± 0.04
B	Vehicle[2]		48	0.6 ± 0.9	0.91 ± 0.08
C	Carvone	1000	24	0.6 ± 0.5	0.97 ± 0.06
D	Carvone	1000	48	1.0 ± 0.7	0.83 ± 0.05
E	CP[3]	50	48	10.4 ± 3.0*	0.34 ± 0.13
Females					
A	Vehicle[2]		24	0.2 ± 0.4	0.99 ± 0.06
B	Vehicle		48	0.4 ± 0.5	1.0 ± 0.09
C	Carvone	1000	24	0.6 ± 0.9	0.95 ± 0.04
D	Carvone	1000	48	0.4 ± 0.5	0.89 ± 0.06
E	CP[3]	50	48	7.2 ± 0.8*	0.63 ± 0.16

1) Five animals per treatment group
2) Corn oil
3) Positive control (cyclophosphamide)
* Significantly different from corresponding control group

An in vivo UDS assay in the liver of male rats with d-carvone was performed in accordance with OECD guideline 468. Doses of 0 (corn oil), 500, 1000, 5000 mg/kg bw were administered by oral gavage. Animals were sacrificed 2-4 or 12-16 h after dosing. Immediately after dosing all animals of the 2000 mg/kg bw group were lethargic. Prior to perfusion at 2-4 or 12-16h after dosing all animals of the 2000 mg/kg bw group had a hunched posture and 4 animals had a rough coat. All

animals of the 1000 mg/kg bw group had a hunched posture and one animal had a rough coat.
No increase in net nuclear grain count was observed in livers of rats treated with carvone. Carvone did not induce unscheduled DNA synthesis in the test.

4.9.2 Human information

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

Positive results have been reported in one chromosome aberration in vitro test with human lymphocytes and one sister chromatid exchanges in vitro CHO cells test. Equivocal results have been observed in a gene mutation test with mouse lymphoma cells and chromosome aberration test with CHO cells. Two Ames tests, one in vivo micronucleus test, and one in vivo UDS assay are negative. The in vivo tests results overrule the positive in vitro findings with respect to chromosome aberrations and SCE. Based on these results carvone is considered to be non-genotoxic.

4.9.5 Comparison with criteria

The classification for mutagenicity is based on the total weight of evidence available, with positive results in somatic cell mutagenicity tests in vivo. As the in vivo micronucleus and UDS tests in mice and in rats are negative, it seems no need for classification for mutagenicity. Carvone does not fulfil the criteria (both CLP and DSD) for classification for mutagenicity.

4.9.6 Conclusions on classification and labelling

Carvone is considered to be non-genotoxic. There is therefore no need to classify Carvone for mutagenicity.

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.

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

NTP performed a carcinogenicity study in mice according to a protocol resembling OECD test guideline 451. In this study mice (50/sex/dose) received 0, 375, or 750 mg d-carvone/kg bw/day by oral gavage. Food intake and haematological analysis were not performed. The results are summarized in the following table.

Table 20: Summary table of the carcinogenicity study

Dose (mg/kg bw/day)	0		375		750		dose related
	m	f	m	f	m	f	
Mortality	13/50	36/50	8/50	21/50	14/50	12/50	
Mean survival (days)	679	639	694	652	631	676	
Clinical signs	no toxicologically relevant effects						
Body weight gain	no toxicologically relevant effects						

Histopathology							
-forestomach							
acanthosis, focal	2/48	5/47	3/48	2/47	5/47	7/49	
acanthosis, multifocal	2/48	1/47	2/48	0/47	6/47	0/49	
-Nasal cavity							
glands hyperplasia	3/50	19/49	42/50 *	45/49 *	44/49 *	49/50 *	dr
atrophy olfact. epithelium	11/50	25/49	42/50 *	46/49 *	44/49 *	49/50 *	dr
acute multifocal							
inflammation turbinate	0/50	5/49	3/50	22/49 *	27/49 *	39/50 *	dr
-Kidney							
chronic focal inflammation	2/50	1/50	5/50	2/50	7/49	4/50	dr
-Rectum							
acute focal inflammation	19/48	5/47	15/45	15/45	18/44	22/45	dr (f)
-Uterus							
dilatation	-	5/50	-	7/50	-	14/50	dr (f)
endometrium hyperplasia	-	14/50	-	26/50	-	27/50	dr (f)
-Lymph node, mesenteric							
multifocal lymph. hyperplas.	11/50	2/46	7/50	3/47	10/48	14/48	(f)
-Spleen							
diffuse lymph. hyperplasia	4/50	4/50	2/50	3/49	2/48	16/50	(f)
multifocal lymph. hyperplas.	12/50	1/50	11/50	2/49	10/48	3/50	
Tumor incidence (see also below)	No toxicological relevant effects						

m/f = male/female, i/d = increased/decreased, is/ds = increased/decreased significantly, np = not performed, a/r = absolute/relative, dr = dose-related. The probability of survival was estimated by the procedure of Kaplan and Meier. Life Table tests, Logistic regression tests, Cochran-Armitage test and Fisher Exact test were applied to assess significance.

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 (not presented in the table above). The lesions in the nose were associated with the presence of foreign material, presumably the corn oil vehicle, which consisted of accumulations of pale yellow foamy or vacuolated material (sometimes with inflammatory exsudate). It is possible that the lesions in the nasal mucosa are due to reflux of the gavage material into the nose after the gavage needle was withdrawn.

In addition to these observations, various dose-related effects (especially in females) were observed at histopathological analysis. Some of these effects were already evident at the low dose (e.g. inflammation of the rectum in females, hyperplasia of the uterus). Although the high mortality rate in female controls may have influenced these results it cannot be excluded that these effects are to some extent due to treatment. Therefore, the dose of 375 mg/kg bw/day is considered to be an effect level. In males, no increase of any type of tumour was observed (see table 21). The overall incidence of neoplasms in females was slightly higher in the treated groups. It is likely that this may be related to lower incidences in the female control group due to the high (early) mortality rate in this group. Moreover, there was no difference in neoplastic incidences between the two treatment groups indicating the absence of any dose-relationship. Therefore, it is concluded that the data do not suggest any carcinogenic effect of d-carvone. Based on dose-related increases in histopathological changes in various organs, the lowest dose used in this study is considered to be an effect level (LOAEL is 375 mg/kg bw/day). A NOAEL cannot be established in this study. Under the conditions of this 2-yr gavage study, there was no evidence of carcinogenic activity of d-carvone.

Table 21: Summary of the incidence of neoplasms

Dose (mg/kg bw)	**0**		**375**		**750**	
	m	**f**	**m**	**f**	**m**	**f**
Liver	50	50	50	50	49	50
Hepatocellular carcinoma	5	0	3	2	3	1
Hepatocellular adenoma	2	1	4	0	4	0
Lymphoma malignant mixed	2	0	0	3	1	3
Stomach, forestomach	48	47	48	47	47	49
Papilloma squamous	1	0	1	3	0	0
Uterus	-	50	-	50	-	50
Polyp	-	0	-	0	-	2
Lymph node, mesenteric	50	46	50	47	49	48
Lymphoma malignant mixed	1	0	1	3	1	2
Spleen	50	50	50	49	48	50
Lymphoma malignant mixed	2	1	1	4	1	4
Skin	50	50	50	50	50	50
Back, subcutaneous tissue, fibroma	2	0	0	0	1	0
Subcutaneous tissue, neurofibrosarcoma	2	0	1	0	0	0
Subcutaneous tissue, sarcoma	4	0	0	1	2	0
Lung	50	50	50	50	50	50
Alveolar/bronchiolar adenoma	7	1	4	6	5	3
Harderian gland	50	50	50	50	50	50
adenoma	1	2	2	0	1	0
Kidney	50	50	50	50	49	50
Lymphoma malignant mixed	1	0	0	1	0	2
Multiple organs	50	50	50	50	50	50
Hemangioma	2	0	0	0	0	0
Lymphoma malignant mixed	4	1	1	4	1	4

Only neoplasms with an incidence >1/sex/dose are included in the table (NTP, 1990).

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No data available.

4.10.3 Other relevant information

No data available.

4.10.4 Summary and discussion of carcinogenicity

A carcinogenicity study was performed in mice. Various dose-related effects (especially in females) were observed at histopathological analysis. Some of these effects were already evident at the 375 mg/kg bw/day (e.g. inflammation of the rectum in females, hyperplasia of the uterus). The dose of 375 mg/kg bw/day is considered to be an effect level. In males, no increase of any type of tumour was observed. The overall incidence of neoplasms in females was slightly higher in the treated groups. It is likely that this may be related to lower incidences in the female control group due to the high (early) mortality rate in this group. Moreover, there was no difference in neoplastic incidences between the two treatment groups indicating the absence of any dose-relationship. Under the conditions of this 2-yr gavage study, there was no evidence of carcinogenic activity of d-carvone.

4.10.5 Comparison with criteria

Classification for carcinogenicity should be on the basis of evidence obtained from animal studies. Under the conditions of the 2-yr gavage study, there was no evidence of carcinogenic activity of d-carvone.

4.10.6 Conclusions on classification and labelling

There is no need to classify Carvone for carcinogenicity.

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.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

One 2 generation study was performed with d-carvone in accordance with OECD guideline 416. The test substance was administered orally (gavage) to rats of the F0 generation at doses of 0, 3, 10 and 30 mg/kg bw/day, starting 10 weeks prior to mating. The treatment of the F1 generation with 90 mg/kg bw/day started when the animals were 3-5 weeks old. The results are shown in the following table.

Table 22: Summary table of the 2 generation study

	Dose (mg/kg bw/day)	0		3		10		30		90		dr
	Sex	m	f	m	f	m	f	m	f	m	f	
F0 animals	Mortality[A]	2	1		1		1		1			
	Clinical signs	no toxicologically relevant effect										
	Body weight (gain)[B]								ds			
	Food consumption[C]								ds			
	Mating, fertility, gestation	no toxicologically relevant effect										
	Oestrus cycle	no toxicologically relevant effect										
	Sperm parameters	no toxicologically relevant effect										

	Organ weights - kidney - thyroids					is[a]		is[r]				
	Pathology											
	- macroscopy	no toxicologically relevant effect										
	- microscopy[D]	no toxicologically relevant effect										
F1 pups	Litter size	no toxicologically relevant effect										
	Survival index	no toxicologically relevant effect										
	Sex ratio	no toxicologically relevant effect										
	Body weight	no toxicologically relevant effect										
	Organ weight	no toxicologically relevant effect										
	Pathology											
	- macroscopy	no toxicologically relevant effect										
	- microscopy (weanlings)	not performed										
F1 animals	Mortality[A]		1				1		2		1	
	Clinical signs					no toxicologically relevant effect						
	Body weightE					no toxicologically relevant effect						
	Food consumption					no toxicologically relevant effect						
	Mating, fertility, gestation					no toxicologically relevant effect						
	Oestrus cycle					no toxicologically relevant effect						
	Sperm parameters					no toxicologically relevant effect						
	Organ weights - liver - kidney							is[r] is[ar]		is[r] is[ar]		dr dr
	Pathology											
	- macroscopy					no toxicologically relevant effect						
	- microscopy[D]					no toxicologically relevant effect						
F2 pups	Litter size					no toxicologically relevant effect						
	Survival index					no toxicologically relevant effect						
	Sex ratio					no toxicologically relevant effect						
	Body weight					no toxicologically relevant effect						
	Pathology											
	- macroscopy					no toxicologically relevant effect						
	- microscopy					not performed						

dr = dose related; i = increased; d = decreased; is = increased significantly; ds = decreased significantly, a= absolute, r=relative

A Animals were found dead or killed in extremis due to bad health or delivery difficulties. The deaths are not considered to be treatment-related.

B Females of the 30 mg/kg bw/day group showed small but significant decreases in body weight gain during lactation on days 4, 7 and 14, and body weight loss on day 4 during lactation.

C Females of the 30 mg/kg bw/day group showed a significant decrease in food consumption from day 1-4 during lactation.

D Males of all treated groups showed histopathological changes in the kidney consistent with accumulation of α_{2u}-globulin.

E Females of the 90 mg/kg bw/day group had in increased body weight and body weight gain. The study authors considers this finding not toxicologically relevant. The present reviewers endorse this view

The effects of carvone on kidneys of male rats are consistent with the carvone-related induction of α_{2u}-globulin accumulation as was demonstrated in a 90-days toxicity study (see 4.7.1.1), and are not considered to be toxicologically relevant for humans. The reduction in body weight and body weight gain in females of the F0 generation treated with 30 mg/kg bw/day is considered not of toxicological significance since no such effects were observed in females of the F1 generation treated with carvone at 30 or 90 mg/kg bw/day. In F1 males treated with carvone at 90 mg/kg bw/day a statistically significant increase in relative liver weight (15%) was observed and considered toxicologically relevant. However, histopathological evaluation on liver of these animals was not performed. The statistically significant increase in relative liver weight in F1 males of the 30 mg/kg bw/day group was small (5%) and is not considered toxicologically relevant. Based on these observations the NOAEL for systemic toxicity is 30 mg/kg bw/day. No effects of carvone on reproductive parameters were observed. Based on this the NOAEL for reproductive effects for treatment of two generations of rats with carvone is 90 mg/kg bw/day.

4.11.1.2 Human information

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

A teratogenicity study was performed with d-carvone in accordance with OECD guideline 414. Female rats were treated on GD 6-20 with 0, 20, 70 and 200 mg/kg bw/day. In addition, acetylcholinesterase (AChE) activity was determined in brain (dams and fetuses) and plasma (dams), collected at gestation day 21. The results are shown in the following table.

Table 23: Summary table of the developmental toxicity study

	Dose (mg/kg bw/day)	0	20	70	200	dr
Maternal effects	Mortality [A]			1		
	Clinical signs	no toxicologically relevant effect				
	Pregnant animals	no toxicologically relevant effect				
	Abortions[A]			1		
	Corpus lutea	no toxicologically relevant effect				
	Body weight gain [B]	no toxicologically relevant effect				
	Food consumption[C]					
	Pathology					
	- macroscopy	no toxicologically relevant effect				
	- microscopy	no toxicologically relevant effect				
Litter response	Live fetuses	no toxicologically relevant effect				
	Fetal weight	no toxicologically relevant effect				
	Pre implantation loss	no toxicologically relevant effect				
	Post implantation loss[D]	no toxicologically relevant effect				
	Sex ratio	no toxicologically relevant effect				
Fetus examination	No. of foetuses	no toxicologically relevant effect				
	No. of abnormal foetuses	no toxicologically relevant effect				
	No. of dead foetuses[D]	no toxicologically relevant effect				
	Malformations					
	External observations and visceral deviations	no toxicologically relevant effect				
	Skeletal deviations	no toxicologically relevant effect				

[A] One animal in the mid-dose group showed an early delivery on GD 19 and was killed.

[B] Body weight gain was statistically significantly reduced on GD 9 and 12 in the mid-dose group and on GD 9, 12 and 15 in the high-dose group. However, the effects were small and are not considered toxicologically relevant

[C] Food consumption in the highest-dose group was decreased from days 6-12 post coitum. Food consumption of the mid-dose group was decreased from GD6-9. However, the reductions were small and not considered toxicologically relevant.

[D] In the highest dose group a significant increase in post implantation loss and number of dead foetuses was observed. This was due to one litter consisting of ten dead foetuses. The study authors consider this a chance finding. The present reviewers endorse this view.

No toxicologically relevant effects were observed. No firm conclusions on the effect of carvone on brain and plasma AChE activity can be drawn from the present study. The highest dose of 200 mg/kg bw/day is considered as the NOAEL for maternal as well as developmental toxicity.

4.11.2.2 Human information

No data available.

4.11.3 Other relevant information

No data available.

4.11.4 Summary and discussion of reproductive toxicity

In the 2 generation study in rats, performed in accordance with OECD guideline 416, no reproductive effects were observed in rats treated with d-carvone at 30 mg/kg bw/day for two generations. In the teratogenicity study in rats, performed in accordance with OECD guideline 414, no maternal or foetal toxicity was observed after treatment with d-carvone at doses up to and including 200 mg/kg bw/day.

4.11.5 Comparison with criteria

Classification for reproductive toxicity is based on the effects that have the potential to interfere with sexual function and fertility as well as the development of the offspring. No reproductive effects as well as no maternal or foetal toxicity are observed in two-generation test and developmental toxicity test.

4.11.6 Conclusions on classification and labelling

There is no need to classify carvone for reproductive toxicity.

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.

Supplemental information - In depth analyses by RAC
Analyses

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Extracts from various Mentha species were analysed for their essential oil contents and were tested for AChE inhibition in vitro. Inhibition of AChE from bovine erythrocytes (0.04 Units/ml) was determined essentially according to the Ellman method. The results show that essential oils from *M. spicata* (> 60% l-carvone) and *M. gentilis* (> 70% l-carvone) as well as l-carvone itself were able to inhibit AChE activity to a moderate extend in vitro. The IC_{50} for AChE activity inhibition in vitro is about 164 µg/ml for l-carvone.
In a second study, the inhibition of AChE from electric eel in vitro was studied for several monoterpene derivates using a method published by Hestrin (not specified). All tested derivates were found to inhibit AChE to some extent, the IC_{50} for d-carvone being 8×10^{-4} M (0.8 mM), equivalent to 120 µg/ml.
In the new developmental study in the rat (4.11.2.1), AChE activity was measured in brains of dams and foetuses and in plasma of dams at gestation day 21. No effect of d-carvone treatment on AChE levels was observed. It has to be noted that brain and plasma were sampled one day after the last d-carvone administration. It cannot be excluded that at this time point brain and plasma levels of d-carvone were low. Since recovery of AChE activity may occur, no firm conclusions on the effect of d-carvone can be drawn from this study alone.

4.12.1.2 Immunotoxicity

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

4.12.2 Summary and discussion

In in vitro experiments, l-carvone as well as Mentha species extracts containing l-carvone were shown to inhibit bovine erythrocyte AChE activity to a moderate extent, the IC_{50} being 164 µg/ml. D-carvone showed an inhibition of AChE from electric eel in vitro, the IC50 being 120 ug/ml. No

effect of d-carvone treatment on AChE levels was observed in vivo. Since recovery of AChE activity may occur in this study, no firm conclusions on the effect of d-carvone can be drawn from this study alone.

4.12.3 Comparison with criteria

In CLP, neurotoxicity should be classified under the category of specific target organ toxicity - repeated exposure, on the basis of animal experiments or epidemiological studies. No effect of d-carvone treatment on AChE levels was observed in vivo.

4.12.4 Conclusions on classification and labelling

The negative results for neurotoxicity in vivo show no need for classification.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

5.1.1 Stability

The determination of the hydrolysis as a function of pH was performed according to OECD 111. At pH 4, 7, and 9, a decrease in concentration <10% was observed after 5 days at 50^0C. It is concluded that carvone is hydrolytically stable.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

A ready biodegradability test was performed with carvone at 1 and 3 mg/l in a closed bottle test (OECD301D). The sampling time interval was 7 days. Within the estimated 10-days window, 51 and 47% degradation was reached, respectively. After 28 days the degradation was 68 and 62%, respectively, with > 60% degradation achieved within a 14-days window. Carvone was assumed to be not inhibitory to bacteria in view of the results. Based on the fact that 60% degradation was reached within the 14-day window, carvone is considered readily biodegradable (EU DAR 2000, EU, 2007).

5.1.2.3 Simulation tests

No data available.

5.1.3 Summary and discussion of degradation

Carvone is hydrolytically stable. The substance is readily biodegradable. Information concerning metabolites of carvone is not available.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

No data available.

5.2.2 Volatilisation

Carvone is volatile (VP = 1.9 ± 0.1 Pa at 25 °C and Henry's Law Constant = 3.6 – 10.6 $Pa.m^3.mol^{-1}$). The variation of the H constant is due to the range in the water solubility: 27-79 mg/l.

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The log Kow of carvone is 2.4, indicating that the substance has a low bioaccumulation potential.

5.3.1.2 Measured bioaccumulation data

No data available.

5.3.2 Summary and discussion of aquatic bioaccumulation

No experimental studies into the bioaccumulation of carvone in fish are available. The log Kow of carvone is 2.4, indicating that the substance has a low bioaccumulation potential.

5.4 Aquatic toxicity

Table 24: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference

OECD 203, Semi-static	96h-LC50, 67 mg/l	zebrafish	DAR, 2000
OECD 202, static	48h-EC50, 46 mg/l	daphnia	DAR, 2000
OECD 201, static	96h ErC50, 41 mg/l 96h NOErC, 11 mg/l	algae	DAR, 2000
Semi-static	EC50, 52 mg/l NOEC, 10 mg/l	*Lemna minor with 8 days of exposure*	DAR, 2005

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

A semi-static zebrafish toxicity test, with daily renewal of test solutions, was performed with five concentrations of 10, 18, 32, 56, and 100 mg/l, plus water control in accordance with OECD 203. Ten fish per vessel and two vessels per concentration were employed. Test vessels were slightly aerated. Samples of the control medium and test solutions of 10, 32 and 100 mg/l were measured at the start of the test and t=24h, except a sampling of the 100 mg/l at t=2h (instead of t= 24h) because all fish were dead at that time. At 100 mg/l actual concentrations were 95 and 56% of nominal at t = 0 and 2 hours; all fish had died at 2 hours. At 10 and 32 mg/l actual concentrations were 95% of nominal at t = 0. At t = 24h, at 10 and 32 mg/l the actual concentrations were 74% and 80% of nominal respectively. At 96h, 2 fish had died at the nominal concentration of 56 mg/l. The re-calculated LC50, based on the raw data, was 67 mg/l (95% confidence interval 56-77 mg/l) expressed as a nominal concentration.

5.4.1.2 Long-term toxicity to fish

No data available.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

A static Daphnia toxicity test was performed with five concentrations of 10, 18, 32, 56, and 100 mg/l, plus water control in accordance with OECD 202. Actual concentrations were 92-103% (average 98% of nominal) at t=0. At t = 48h, at 10, 32 and 100 mg/l, actual concentrations were 72, 88 and 87% of nominal, respectively. The reported 48-hours EC50 is 46 mg/l (95% confidence interval 41-53 mg/l) expressed as nominal concentration.

5.4.2.2 Long-term toxicity to aquatic invertebrates

No data available.

5.4.3 Algae and aquatic plants

An algae toxicity test with *Selenastrum capricorrutum* was performed with six concentrations, 3.3, 11, 18, 32, 46 and 99 mg/l, plus control in accordance with OECD 201. Actual concentrations were 97-101% at t = 0, and 69% at 18 and 99 mg/l after 96 hours. An EC50 for growth rate of 41 mg/l

(95% confidence interval 37.8-44.3 mg/l) was calculated. For biomass these values are 26 mg/l (18-32 mg/l). A NOEC for growth rate of 11 mg/l is estimated.

A toxicity test with *Lemna minor* was performed with a nominal concentration range of 4.6, 10, 22, 46 and 100 mg test substance/L in accordance with ISO standard proposal: "water quality – Duckweed growth inhibition test" (2000) and a draft OECD guideline: "*Lemna* sp. growth inhibition test" (1999). The reference substance was 3,5-dichlorophenol. Test solutions were renewed every 2 days. A SIS-medium was used as the test medium. Three replicates were used per concentration with exception of the highest concentration, which had six replicates. In the untreated control, six replicates were used. Each replicate consisted of four plants with 10 fronds per vessel. Actual concentrations were measured at days 0, 2 and 6 in duplicate samples. Test was performed under continuous lighting with light intensity 85-96 μE m^{-2} s^{-1}. Frond numbers were counted at the start, after 4, 6 days and at the end of the test of 8 days. Numbers of affected fronds were recorded at the same time-points. Numbers of affected fronds were recorded at the same time-points. Average growth rate at each test substance concentration was compared with the control value and the percentage reduction in growth was calculated. The mean analytical recovery was 98.1%. Mean actual recoveries were between 91 and 98% of nominal in samples in the period 0-2 days. At day 6 concentrations were above 84%, except at the lowest level (72%). EC_{50} values for the periods 0-4, 0-6 and 0-8 days were 52 mg/L, 65 mg/L and 75 mg/L, respectively. The NOEC value was 10 mg/l (EU DAR, addendum, 2005).

5.4.4 Other aquatic organisms (including sediment)

No data available.

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

Conclusion of environmental classification according to Directive 67/548/EEC

Carvone is readily biodegradable. The log Kow is < 3, indicating that the substance has a low bioaccumulation potential. The L(E)C50 values for fish, Daphnia, algae and aquatic plant are between 10 – 100 mg/l. Therefore, carvone does not fulfill the criteria for classification following Directive 67/548/EEC

Conclusion of environmental classification according to Regulation EC 1272/2008

Carvone is rapidly biodegradable. The log Kow is < 4, indicating that the substance has a low bioaccumulation potential. The L(E)C50 values for fish, Daphnia, algae and aquatic plant are between 10 – 100 mg/l. Furthermore, the available NOEC values are > 1 mg/l. Therefore, carvone does not fulfil the criteria for classification following Regulation EC 1272/2008.

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

Carvone does not need to be classified for the environment according to Directive 67/548/EEC and Regulation EC 1272/2008.

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 $\pm$20 per cent 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.

6 OTHER INFORMATION

7 REFERENCES

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8 ANNEXES