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# Carvone

## Toxicity monograph

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# Carvone

# Toxicity monograph

## INTRODUCTION

(b) (4) was asked to produce a toxicity monograph of carvone (CAS RN[1] 99-49-0), focussing on the inhalation route of exposure. Data on the inhalation of tobacco smoke containing the ingredient (if available) have not been included in this monograph.

## EXPERTISE

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## TOXICITY DATA SEARCH CRITERIA

Searches of (b) (4) (see Appendix for details) identified several recent and relevant expert group reports that formed the basis for this assessment, notably ECHA (2012) and JECFA (1999) (which included key data on L- and D-carvone as well as carvone with no specified isomeric composition). A subsequent search of the primary literature was restricted to (b) (4) and Toxline (the toxicity subset of Medline, via TOXNET) in an attempt to identify more recent data since the ECHA review. The remainder of the TOXNET system (which includes HSDB, GENETOX, DART, CCRIS, IRIS, ITER and CPDB) was also consulted. Since the key review could not necessarily be relied upon specifically to identify cardiopulmonary data, and to ensure all critical local and systemic inhalation data were identified, no date restriction was placed on searches in PubMed tailored to identify such information.

All searches were conducted in September 2016 using the CAS RN(s) and (in PubMed only) name identified below, as appropriate.

The data summarised in this report refers to the unheated form unless otherwise stated.

[1] Chemical Abstracts Service Registry Number.

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## IDENTIFICATION, REACH STATUS AND EU CLASSIFICATION

| Identifier / status | | | |
|---|---|---|---|
| Name[3] | Carvone[4] | L-carvone | D-carvone |
| Synonym(s) | 2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one<br>p-Mentha-6,8-dien-2-one | (-)-Carvone<br>(R)-Carvone<br>(R)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one<br>(R)-p-Mentha-6,8-dien-2-one | (+)-Carvone<br>(S)-Carvone<br>(S)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one<br>(S)-p-Mentha-6,8-dien-2-one |
| CAS RN | 99-49-0 | 6485-40-1 | 2244-16-8 |
| REACH registration number[5] | Not REACH registered | 01-2119962458-25-xxxx | Not REACH registered |
| Classification, according to EU CLP (EC 1272/2008)[6] | Harmonised classification: | | |
| | Skin Sens. 1 (H317; may cause an allergic skin reaction) | | |

## TOXICOLOGY

### LOCAL EFFECTS

#### Respiratory tract irritation

Clinical signs reported in an OECD guideline study, in which rats [group size unspecified] were exposed to an aerosol of carvone (with a D/L isomer ratio of ≥4:1) at 5.66 g/m³ [presumably for 4 hours] and observed for 2 weeks, did not include indications of irritation (cited in ECHA, 2012, 2013a). Since no direct indications of irritation were observed in the acute inhalation study, carvone does not meet the criteria for classification as a respiratory tract irritant (ECHA, 2012, 2013a). [See also Single dose toxicity and Cardiopulmonary effects sections.]

#### Skin irritation

Human

L-Carvone tested at 1% in petrolatum produced no skin irritation following a 48-hour closed patch test in 25 subjects (Kligman, 1971). Similarly, no irritation was observed in subjects patch tested with D-carvone (2% in petrolatum) for 48 hours (Kligman, 1976). [See also skin sensitisation section.]

[3] It is noted that various nomenclature options exist for carvone (R/S; L/D; +/-). For the purposes of consistency, the enantiomers will be referred using the L/D nomenclature for the remainder of the report (irrespective of the name listed in the reference).

[4] Isomeric mixture or isomeric composition not specified/unknown.

[5] REACH registration numbers are substance and company specific. Therefore, the substance-specific part of the registration number is included here, from data disseminated on the ECHA 'registered substance' website. The REACH dossier has not been consulted for toxicity data.

[6] Recommended for harmonised classification as a skin sensitiser.

Non-human

Neat D-carvone produced transient erythema in rabbits following a 24-hour occlusive application to intact or abraded skin (Levenstein, 1976). In a guideline study, carvone (isomers not specified) was found to be mildly irritating to rabbit skin [dose unspecified but probably 0.5 mL of neat test material] (cited in ECHA, 2012, 2013a).

### Eye irritation

Human

No substance-specific data were identified for either isomer (or the mixture/unspecified form).

Non-human

No substance-specific data were identified for D-carvone.

Undiluted L-carvone (0.1 mL) was not irritating to the eyes of 4 female rabbits, examined after 24 hours after instillation (Anon., 1999b). However, in a guideline study, carvone (isomers not specified) was found to be mildly irritating to the rabbit eye [dose unspecified but probably 0.1 mL of neat test material] (cited in ECHA, 2012, 2013a).

### Other local effects

No substance-specific data were identified for either isomer (or the mixture/unspecified form).

## SENSITISATION AND INTOLERANCE

### Respiratory tract sensitisation

No substance-specific data were identified for either isomer (or the mixture/unspecified form).

### Skin sensitisation

The Danish EPA has submitted a proposal for a harmonised classification as a skin sensitiser Category 1, or sub-category 1A or 1B, for carvone (Danish EPA, 2016).

The ECHA Committee for Risk Assessment (RAC) concluded that carvone (and its stereoisomers) should be classified as a skin sensitiser category 1 (ECHA, 2013b).

Human

In a maximisation[7] test, application of L-carvone (1% in petrolatum) produced no skin sensitisation reactions in a group of 25 subjects (Kligman, 1971). Similarly, no sensitisation was observed in a maximisation test with D-carvone (2% in petrolatum) on 25 subjects (Kligman, 1976). [See also skin irritation section.]

[7] The test procedure typically involves an initial induction phase of five 48-hour covered patch tests, followed 10-14 days later by a 48-hour covered challenge patch.

Patch tests with L-carvone (5% in petrolatum) induced positive reactions in 15 out of 541 eczema patients (2.8%). Surprisingly, upon re-testing a subgroup of the 15 suspected carvone-allergic patients (with compound concentrations of 1, 2 and 5% in petrolatum), only 2 of 8 patients produced a positive skin response (Paulsen *et al.*, 1993).

Several case studies are available in which patch tests with carvone isomers (generally as components of consumer products) were positive[8].

Non-human

In a LLNA[9], female mice (4/group) received dermal applications (to the ear) of L-carvone at concentrations of 2.5-50% on 3 consecutive days. SI[10] values were calculated on study day 6. The EC3 value[11] was 10.7% (RIFM, 2007a), indicative of a weak sensitiser. In an apparent repeat of the above assay [same group size and test concentrations, presumably also using female mice] a lower EC3 value of 5.7% was calculated [no further details in citing source] (RIFM, 2007b), indicative of a moderate sensitiser. Higher EC3 values have been calculated in various other studies.

On the basis of a GPMT[12] investigation with carvone (isomer ratio unspecified), the compound was considered to be a skin sensitiser (ECHA, 2012, 2013a).

**Oral allergy/intolerance**

No substance-specific data were identified for either isomer (or the mixture/unspecified form).

**INHALATION TOXICITY STUDIES**

**Single dose toxicity**

Human

No substance-specific data were identified for either isomer (or the mixture/unspecified form).

Non-human

No enantiomer-specific data were identified.

In an OECD guideline study, rats [group size unspecified] were exposed to an aerosol of carvone (with a D/L isomer ratio of ≥4:1) at 5.66 g/m$^3$ [presumably for 4 hours] and observed for 2 weeks. One female died the day after the exposure. Clinical signs during exposure included decreased breathing frequency, post-inspiratory apnoea [temporary breathing cessation], superficial breathing, restlessness, stress, incoordination and tremors. Following

[8] Due to time constraints, these have not been summarised in the monograph at this stage.
[9] Local Lymph Node Assay.
[10] Stimulation index.
[11] OECD test guideline 429 defines stimulation index (SI) as the ratio of lymphocyte proliferation in a treated group to that in the vehicle control group. An SI of ≥3 is considered positive and the estimated concentration three (EC3) is the estimated concentration of a test substance needed to produce an SI of 3. ECETOC definitions of potency are: extreme sensitizer (EC3 value <0.1); strong sensitizer (EC3 >0.1 - ≤1); moderate sensitizer (EC3 value >1 - <10; and weak sensitizer (EC3 value >10).
[12] Guinea Pig Maximisation Test.

exposure, increased breathing frequency, post-inspiratory apnoea and dyspnoea [shortness of breath] were seen. Dirty and wet fur was observed 24-48 hours after treatment and alopecia was observed in a few rats at days 7-13. Growth was impaired in most rats during the first week after treatment, returning to normal in the second week (except for two females). 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 $LC_{50}$ value[13] was >5.66 g/m³ (cited in ECHA, 2012, 2013a), which is indicative of a low degree of acute inhalation toxicity. [See also Respiratory tract irritation and Cardiopulmonary effects sections.]

### Repeated dose toxicity

No substance-specific data were identified for either isomer (or the mixture/unspecified form).

## TOXICITY STUDIES – OTHER EXPOSURE ROUTES

An EFSA Scientific Committee noted that the acute toxicity data suggest that L-carvone is not more toxic than D-carvone (EFSA, 2014a).

### Single dose toxicity

Human

No substance-specific data were identified for either isomer (or the mixture/unspecified form).

Non-human

The acute oral $LD_{50}$ value[14] for L-carvone in rats was reported to be 5900 mg/kg bw in males and 4900 mg/kg bw in females (Anon., 1986). For D-carvone, a value of 3710 mg/kg bw was noted in rats (Levenstein, 1976). Values of 1640 and 766 mg/kg bw were reported for carvone (unspecified) in rats and guinea pigs respectively (Jenner *et al.*, 1964); a value of ">2000 mg/kg bw" was subsequently established in rats (cited in ECHA, 2012, 2013a).

The dermal $LD_{50}$ value for L-carvone was in excess of the limit dose of 2000 mg/kg bw in rats (Anon., 1999a). Similarly, a value of 4 mL/kg [equivalent to 3860 mg/kg bw (JECFA, 1991)] was reported for D-carvone in rabbits (Levenstein, 1976).

Slow breathing was apparent in mice receiving an intraperitoneal injection of carvone (isomer ratio unspecified) at 1000 mg/kg bw (cited in ECHA, 2012, 2013a). [See also Genotoxicity and Cardiopulmonary effects sections.]

### Repeated dose toxicity

Human

No substance-specific data were identified for either isomer (or the mixture/unspecified form).

[13] Lethal Concentration 50, i.e. the concentration that is lethal to 50% of the exposed group.
[14] Lethal Dose 50, i.e. the dose that is lethal to 50% of the exposed group.

Non-human

The repeated dose toxicity profile of carvone has been well studied by the oral route (the good-quality data mainly relate to D-carvone).

In subacute studies carvone (isomer unspecified) was administered via the oral route to rats for 14 days at 0, 50, 200 or 1000 mg/kg bw/day and to mice for 16 days at 0, 150, 328, 723, 1590 or 3000 mg/kg bw/day. At 1000 mg /kg bw/day in rats, and from 1590 mg/kg bw/day in mice, all the animals died. In rats, clinical signs of toxicity and a significant increase in relative kidney weights in males were observed, and slight effects on haematological and biochemical parameters were noted, from 200 mg/kg bw/day. No adverse effects were reported at 50 mg/kg bw/day, the study NOAEL[15]. In mice, overt signs of toxicity were observed from 723 mg/kg bw/day. Relative liver weights were increased and thymus weights had decreased in all treated groups, hence the study LOAEL[16] was 150 mg/kg bw/day (the lowest tested dose) (cited in ECHA, 2012, 2013a).

Significant increases in serum cholesterol and triacylglycerol concentrations were seen in four male rats fed carvone (unspecified stereochemistry) in the diet at 1% (equivalent to 500 mg/kg bw/day) for 14 days, compared with the controls. Significant decreases in food consumption and terminal body weights were also reported in treated animals (Imaizumi et al., 1985). [No macro- or microscopic examination appears to have been undertaken.]

A subchronic NTP study involved the gavage administration of D-carvone to rats (10/sex/group) at doses of 0, 93, 187, 375, 750 or 1500 mg/kg bw/day. A detailed examination was conducted[17]. Most animals from the two highest dose groups died during the study. Reduced growth was only apparent in males at 187 and 375 mg/kg bw/day. Increased relative liver weight was observed in both sexes at 93 mg/kg bw/day and above. An increase in relative weight of the other organs was seen at higher doses (NTP, 1982). A NOEL of 93 mg/kg bw/day was reported (JECFA, 1999). [See also Reproductive and developmental toxicity and Cardiopulmonary effects sections.]

In a subsequent 90-day toxicity study, rats (10/sex/group) were administered D-carvone by gavage at doses of 0, 5, 30 or 180 mg/kg bw/day. An extensive assessment was conducted[18]. At 30 mg/kg bw/day, observations included increases in relative liver (females) and kidney weights as well as various haematological and biochemical effects. There were no histopathological changes related to treatment apart from those in the kidney which consisted of tubular necrosis, exclusively in male rats, and basophilic tubules in both sexes (Schoenmakers, 1996). Following a re-evaluation of the kidney slides from this study, it was concluded that the renal histopathological lesions observed in male animals of the mid- and high-dose group were diagnosed as $\alpha_{2u}$ globulin nephropathy (Schoenmakers, 2003). "As this protein is not present in higher mammals including man, these $\alpha_{2u}$-globulin-related effects

[15] No-observed-adverse-effect level.

[16] Lowest-observed-adverse-effect level.

[17] Gross pathology was performed on all animals, while the weight of weights of brain, lungs, heart, thymus, liver, right testis and right kidney were recorded. Histopathological examination was conducted on all control rats, those given 375 mg/kg bw/day and all animals that died or were killed before the end of the study.

[18] Including clinical signs of toxicity; haematological and biochemical effects; adrenal, brain, heart, kidney, liver, ovary, prostate, spleen, testis and thymus weights; gross and microscopic examination of a full range of organs and tissues.

can be considered not relevant for exposure risk assessment of carvone in man. The NOAEL in this study is 5 mg/kg bw/day" (ECHA, 2012, 2013a). [See also Cardiopulmonary effects section.]

Rats (5/sex/group) were fed carvone (isomer unspecified but thought by JECFA (1999) to be L-carvone) in the diet at a concentration of 1000 ppm (providing about 50 mg/kg bw/day) for 27-28 weeks, 2500 ppm [about 125 mg/kg bw/day] for 1 year or 10,000 ppm [about 500 mg/kg bw/day] for 16 weeks. Control groups (10/sex) were included for each study period. No adverse effects were observed in rats dosed for 27-28 weeks or 1 year as judged by growth, appearance, food intake, haematology, final body weights, or gross and microscopic examination of the major organs. [Although no effects were noted, only a very limited report of the results of this study was provided.] Depressed growth and testicular atrophy were the only reported effects in the 16-week study (Hagan *et al*., 1967). "The NOEL was 125 mg/kg bw/day", from the 1-year study (JECFA, 1999). [See also Reproductive and developmental toxicity and Cardiopulmonary effects sections.]

In a high-quality chronic investigation conducted under the auspices of the National Toxicology Program, mice (50/sex/group) received D-carvone by gavage, 5 days/week for 103 weeks, at doses of 0, 375 or 750 mg/kg bw/day. Complete histopathological examination[19] was performed on all animals [organs do not appear to have been weighed]. No clinical signs were observed. Growth was not significantly affected in either sex. Interestingly, a dose-related statistically significant decrease in mortality was observed in females. This was attributed largely to a very high death rate in the control females[20]. There were dose-related increased incidences and/or severity of lesions in the nasal cavities of both sexes (probably due to a local effect of D-carvone caused by a reflux of the gavage material when the gavage needle was withdrawn). Atrophy of the olfactory epithelium and hyperplasia of the underlying Bowman's glands occurred together at high incidences in either sex in both dosed groups (NTP, 1990). "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" (ECHA, 2012, 2013a). Subacute and subchronic studies conducted by NTP as precursors to this evaluation did not report any lower NOAELs (NTP, 1990). [See also Carcinogenicity and Cardiopulmonary effects sections.]

## GENOTOXICITY

### Expert-group opinion

D-Carvone was found to be genotoxic *in vitro*. However, it was not carcinogenic in mice. The EFSA CEF Panel[21] concluded that "this substance together with the structurally related L-carvone could be evaluated through the Procedure", indicating a lack of concern with

[19] including the adrenal glands, aorta, brain, epididymis, esophagus, femur, gall bladder, gross lesions, Harderian gland, heart, kidney, large intestine (caecum, colon, rectum), larynx, liver, lung, lymph node (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland , prostate gland, rectum, salivary gland, seminal vesicle, skeletal muscle, skin, small intestine (duodenum, ileum, jejunum), spinal cord, spleen, sternebrae, stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

[20] This was likely related to the high incidence of ovarian abscesses. However, it is uncertain whether the lower incidence of ovarian abscesses in dosed females was related to D-carvone administration.

[21] EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids.

respect to genotoxicity. Based on *in vivo* metabolism studies in humans, metabolism of carvone is not influenced by stereochemistry: "accordingly, the results for D-carvone can be used for L-carvone as well" (EFSA, 2014b).

Carvone and its stereoisomers do not meet the criteria for classification for mutagenicity (ECHA, 2013b).

Mammals (*in vivo*)

No substance-specific data were identified for L-carvone.

D-Carvone did not induce unscheduled DNA synthesis in male rats following oral gavage at up to 2000 mg/kg bw (cited in ECHA, 2012, 2013a).

An *in vivo* micronucleus test involved the intraperitoneal injection of mice (5/sex) with carvone (isomer ratio unspecified) at 1000 mg/kg bw. There was no increase in the frequency of micronucleated cells [peripheral erythrocytes]. The ratio of polychromatic/ normochromatic erythrocytes was slightly, but not significantly, decreased at 48 hours in both sexes. Based on this study, it was concluded that carvone is not genotoxic (cited in ECHA, 2012, 2013a). [See also Toxicity studies – other routes and Cardiopulmonary effects section.]

Mammalian cells (*in vitro*)

No evidence of gene mutations was observed when L-carvone was tested up to cytotoxic concentrations[22] in mouse lymphoma cells in the presence or absence of S9[23] (Anon., 2012a). The compound was not considered to be clastogenic following 4-hour exposure of human lymphocytes (Anon., 2012b).

D-Carvone induced chromosome aberrations in CHO[24] cells in the absence and presence of S9 (NTP, 1990).

Micro-organisms

L-Carvone was not mutagenic in a high-quality bacterial reverse mutation (Ames) test in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 or in *Escherichia coli* strain WP2 urv A when tested at up to 5 mg/plate, both in the presence and absence of S9 (Anon., 2012c).

In another good-quality Ames test, D-carvone was non-mutagenic is *S. typhimurium* strains TA98, TA100, TA1535 and TA1537, when tested up to 333 µg/plate [the cytotoxic limit] with and without S9 (Mortelmans *et al*., 1986).

[22] Up to 372 µg/mL (4 hours, with and without S9), 300 µg/mL (4 hours, with S9) and 100 µg/mL (24 hours, without S9).
[23] Induced mammalian liver post-mitochondrial fraction used for metabolic activation.
[24] Chinese Hamster Ovary.

## CARCINOGENICITY

### Human

No substance-specific data were identified for either isomer (or the mixture/unspecified form).

### Non-human

No increases in tumour incidence were observed in mice (50/sex/dose) administered D-carvone by gavage at up to 750 mg/kg bw/day for 2 years. The incidence of female control mice with primary neoplasms and the total numbers of primary neoplasms were low compared to the dosed groups, though this may be related to the early deaths of female control mice. The incidences of male mice with primary neoplasms and the total numbers of primary neoplasms were significantly lower in dosed groups than in vehicle controls (NTP, 1990). [See also Toxicity studies – Other exposure routes and Cardiopulmonary effects sections.]

In a limited 6-month study, female mice[25] (20/group) received 24 intraperitoneal injections of L-carvone at 50 or 250 mg/kg bw/treatment day (3 times/week for 8 weeks). The liver, kidney, spleen, thymus, intestine, salivary and endocrine glands were grossly examined for abnormalities. Microscopic examination was limited to the liver. No evidence of carcinogenicity was observed (Stoner *et al*., 1973). [Modern guidelines recommend the administration of several dose levels by a physiological route to groups of 50/sex for lifetime followed by histopathological examination of a comprehensive range of tissues and organs.]

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

There is no experimental evidence for fertility impairment or developmental toxicity of carvone (ECHA, 2013b).

### Human

No substance-specific data were identified for either isomer (or the mixture/unspecified form).

### Non-human

An OECD test guideline 416 two-generation reproduction toxicity study was performed involving gavage administration of D-carvone to F0 rats [group sizes unspecified in citing report] at 0, 3, 10 or 30 mg/kg bw/day, starting 10 weeks prior to mating. F1 rats were dosed at 0, 10, 30 or 90 mg/kg bw/day[26] [no further details on the dosing regimen]. Parental toxicity was observed at 30 mg/kg bw/day (and above, where tested: statistically significant decreased growth and food consumption in F0 females, increased relative kidney weight in F0 and F1 males and relative liver weight in F1 males). Males of all treated groups showed histopathological changes in the kidney consistent with accumulation of $\alpha_{2u}$-globulin. The renal effects of D-carvone were not considered to be toxicologically relevant for humans. The growth reduction in F0 females was not considered to be of toxicological significance as this effect was absent in the F1 generation. The slight, but statistically significant increase, in

[25] Of a strain genetically susceptible to lung tumours.

[26] The treatment of F1 rats at the top dose started when the animals were 3-5 weeks old.

relative liver weight in F1 males of the 30 mg/kg bw/day group was not considered toxicologically relevant. No other toxicologically relevant effects were apparent and there were no effects on reproductive parameters (cited in ECHA, 2012, 2013a). "Based on these observations the NOAEL for systemic toxicity is 30 mg/kg bw/day. The NOAEL for reproductive effects for treatment of two generations of rats with carvone is 90 mg/kg bw/day" (ECHA, 2012, 2013a).

In a guideline teratogenicity study[27], female rats were treated with D-carvone at 0, 20, 70 or 200 mg/kg bw/day on gestation day (GD) 6-20 by gavage. Gross and histopathological maternal pathology was conducted, while foetuses were examined for malformations. In addition, acetylcholinesterase (AChE) activity was determined in brain (dams and foetuses) and plasma (dams), collected at GD 21. Statistically significant reductions in growth, and decreased food consumption, were apparent in the mid- and high-dose groups during the study but were not considered toxicologically relevant. There was a statistically significant increase in post implantation loss and number of dead foetuses in the high-dose group due to 1 litter consisting of ten dead foetuses. However, this was considered a chance finding (cited in ECHA, 2012, 2013a). "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" (ECHA, 2012, 2013a).

As part of the NTP 90-day toxicity study in rats, male reproductive function was tested in 5 animals from the groups receiving D-carvone at doses of 0, 93 or 375 mg/kg bw/day (20 additional animals were included in each of these groups for this purpose). Semen was collected and assessed for sperm concentration, motility and morphology. At 375 mg/kg bw/day there was depressed sperm motility and a slight decrease in sperm concentration in the assessments made at the end of the study but not on any other occasion. Similar changes were seen in the only male surviving to the end of the main study from the 750 mg/kg bw/day group, however in all the males from that group there was testicular degeneration and relative aspermia. No effects were seen on testes or sperm parameters or any aspect of reproductive function at lower doses (NTP, 1982). [See also Toxicity studies – Other exposure routes section.]

Testicular atrophy was reported in male rats fed L-carvone in the diet at 500 mg/kg bw/day for 16 weeks (Hagan *et al.*, 1967). [See also Toxicity studies – Other exposure routes section.]

## CARDIOPULMONARY EFFECTS[28]

In an acute inhalation toxicity study, decreased breathing frequency, post-inspiratory apnoea [temporary breathing cessation] and superficial breathing were noted during exposure in rats exposed to carvone (D/L isomer ratio of >=4:1) at 5.66 g/m$^3$ [presumably for 4 hours]. Following exposure, increased breathing frequency, post-inspiratory apnoea and dyspnoea

[27] OECD test guideline 414: Prenatal development toxicity study.
[28] Potential effects on the heart, blood vessels and/or respiratory tract.

[shortness of breath] were seen (cited in ECHA, 2012, 2013a). [See also Inhalation toxicity studies section.]

Slow breathing was apparent in mice receiving an intraperitoneal injection of carvone (isomer ratio unspecified) at 1000 mg/kg bw (cited in ECHA, 2012, 2013a). [See also Toxicity studies – Other routes and Genotoxicity sections.]

There were no treatment-related microscopic lesions detected in the hearts or lungs of mice subject to chronic gavage treatment with D-carvone (at up to 750 mg/kg bw/day). Relative lung/bronchi weights were anomalously[29] decreased in rats exposed to 187 mg/kg bw/day for 13 weeks (NTP, 1990). [See also Toxicity studies – Other exposure routes and Carcinogenicity sections.]

In a subchronic NTP study involving the gavage administration of D-carvone to rats (10/sex/group) at doses of 0, 93, 187, 375, 750 or 1500 mg/kg bw/day, there were no effects on the heart or lungs in a detailed examination (NTP, 1982). [See Toxicity studies – Other exposure routes section for further details.] Similarly, in a subsequent 90-day toxicity study, in which rats (10/sex/group) were administered D-carvone by gavage at doses of 0, 5, 30 or 180 mg/kg bw/day, no effects on the heart or lung were seen (ECHA, 2012, 2013a). [See Toxicity studies – Other exposure routes section for further details.]

Rats (5/sex/group) fed carvone (isomer unspecified but thought by JECFA (1999) to be L-carvone) in the diet at a concentration of 1000 ppm (providing about 50 mg/kg bw/day) for 27-28 weeks, 2500 ppm [about 125 mg/kg bw/day] for 1 year or 10,000 ppm [about 500 mg/kg bw/day] for 16 weeks showed no effects on the heart or lungs (Hagan *et al*., 1967). [See Toxicity studies – Other exposure routes section for further details.]

## REFERENCES

Anon. (1986). Study report [cited as "Acute toxicity: oral" in the ECHA REACH dossier for l-p-mentha-1(6),8-dien-2-one (Wanxiang International (Europe) Limited, 2015)].

Anon. (1999a). Study report [cited as "Acute toxicity: dermal" in the ECHA REACH dossier for l-p-mentha-1(6),8-dien-2-one (Wanxiang International (Europe) Limited, 2015)].

Anon. (1999b). Study report [cited as "Eye irritation" in the ECHA REACH dossier for l-p-mentha-1(6),8-dien-2-one (Wanxiang International (Europe) Limited, 2015)].

Anon. (2012a). Study report [cited as "Exp Key Genetic toxicity in vitro.001" in the ECHA REACH dossier for l-p-mentha-1(6),8-dien-2-one (Wanxiang International (Europe) Limited, 2015)].

Anon. (2012b). Study report [cited as "Exp Key Genetic toxicity in vitro.002" in the ECHA REACH dossier for l-p-mentha-1(6),8-dien-2-one (Wanxiang International (Europe) Limited, 2015)].

[29] No analogous decrease was observed at the remaining doses of up to 750 mg/kg bw/day.

Anon. (2012c). Study report [cited as "Exp Key Genetic toxicity in vitro.003" in the ECHA REACH dossier for l-p-mentha-1(6),8-dien-2-one (Wanxiang International (Europe) Limited, 2015)].

Danish EPA (2016). Environmental Protection Agency, Ministry of Environment and Food of Denmark. Evaluation of selected sensitizing fragrance substances. Environmental project No. 1840, 2016. http://www2.mst.dk/Udgiv/publications/2016/03/978-87-93435-46-9.pdf

ECHA (2012). European Chemicals Agency. Proposal for harmonised classification and labelling on carvone. Based on Regulation (EC) No. 1272/2008 (CLP Regulation), Annex VI, Part 2. CLH report, Version number 3, 21 September 2012. [No bibliographic details provided for the studies cited.] http://echa.europa.eu/documents/10162/06316e80-54ad-455b-9ef2-5774d45aaf99

ECHA (2013a). European Chemicals Agency. Committee for Risk Assessment RAC Annex 1. Background document to the Opinion proposing harmonised classification and labelling at Community level of carvone. Adopted 4 June 2013. [No bibliographic details provided for the studies cited.] https://echa.europa.eu/documents/10162/c54aee6b-1040-4ffd-14c5-cf60171365b4

ECHA (2013b). European Chemicals Agency. Committee for Risk Assessment RAC. Opinion proposing harmonised classification and labelling at EU level of carvone. Adopted 4 June 2013. [No bibliographic details provided for the studies cited.] https://echa.europa.eu/documents/10162/3c6652b8-b91f-5997-7a81-262cdff20a56

EFSA (2012). European Food Safety Authority. EFSA Scientific Committee. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 10(3), 2579, 1-32. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2579.pdf

EFSA (2014a). European Food Safety Authority. EFSA Scientific Committee. Scientific Opinion on the safety assessment of carvone, considering all sources of exposure. EFSA Journal 12(7), 3806, 1-74. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3806.pdf

EFSA (2014b). European Food Safety Authority. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). Scientific Opinion on Flavouring Group Evaluation 212, Revision 2 (FGE.212Rev2): α,β-Unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19. EFSA Journal 12(2):3584, 1-44. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3584.pdf

EMA (2015). European Medicines Agency. Assessment report on *Carum carvi* L., fructus and *Carum carvi* L., aetheroleum. EMA/HMPC/715093/2013. 7 July 2015. http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2015/09/WC500193275.pdf

European Commission (2012). Scientific Committee on Consumer Safety (SCCS). Opinion on fragrance allergens in cosmetic products. Opinion adopted by the SCCS at its 15th plenary meeting of 26-27 June 2012. SCCS/1459/11. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_102.pdf.

Hagan EC, Hansen WH, Fitzhugh OG, Jenner PM, Jones WI, Taylor JM, Long EL, Nelson AA and Brouwer JB (1967). Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. Food and Chemical Toxicology 5, 141-157.

Imaizumi K, Hanada K, Mawatari K and Sugano M (1985). Effect of essential oils on the concentration of serum lipids and apolipoproteins in rats. Agricultural and Biological Chemistry 49, 2795-2796 [cited in JECFA, 1999].

JECFA (1991). Toxicological evaluation of certain food additives and contaminants. Prepared by the thirty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series 28. World Health Organization, Geneva. http://www.inchem.org/documents/jecfa/jecmono/v28je11.htm

JECFA (1999). Safety evaluation of certain food additives. Prepared by the fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series 42. World Health Organization, Geneva. http://www.inchem.org/documents/jecfa/jecmono/v042je18.htm

Jenner PM, Hagan EC, Taylor JM, Cook EL and Fitzhugh OG (1964). Food flavourings and compounds of related structure. I. Acute oral toxicity. Food and Cosmetics Toxicology 2, 327-343 [cited in Opdyke, 1973].

Kligman AM (1971). Report to RIFM, 14 June [cited in Opdyke, 1973].

Kligman AM (1976). Report to RIFM, 9 April [cited in Opdyke, 1978].

Levenstein I (1976). Report to RIFM, 18 August [cited in Opdyke, 1978].

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environmental Mutagenesis 8 (Suppl. 7), 1-119 [cited in NTP, 1990].

NTP (1982). National Toxicology Program. Subchronic oral toxicity test with carvone in rats. Unpublished report [cited in EFSA, 2014a].

NTP (1990). National Toxicology Program. Toxicology and carcinogenesis studies of d-carvone (CAS No. 2244-16-8) in B6C3F1 mice (gavage studies). NTP Technical Report 381. NIH Publication No. 90-2836. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr381.pdf

Opdyke DLJ (1973). Monographs on Fragrance Raw Materials: l-Carvone. Food and Cosmetics Toxicology 11, 1057-1058.

Opdyke DLJ (1978). Monographs on Fragrance Raw Materials. d-Carvone. Food and Cosmetics Toxicology 16, 673-674.

Paulsen E, Andersen KE, Carlsen L and Egsgaard H (1993). Carvone: an overlooked contact allergen cross-reacting with sesquiterpene lactones? Contact Dermatitis 29, 138-143.

RIFM (2007a). Research Institute for Fragrance Materials. Local Lymph Node Assay on carvone. RIFM report number 52902 (RIFM, Woodcliff Lake, NJ, USA) [cited in European Commission, 2012; Wanxiang International (Europe) Limited, 2015].

RIFM (2007b). Research Institute for Fragrance Materials. Local Lymph Node Assay on carvone. RIFM report number 52907 (RIFM, Woodcliff Lake, NJ, USA) [cited in European Commission, 2012].

Schoenmakers ACM (1996). 90-day oral toxicity with Carvone min. 95% by daily gavage in the rat. Generated by NOTOX B.V., 's-Hertogenbosch, The Netherlands. Company file 152426, Makhteshim file TO-681. GLP, Unpublished, Confidential report [cited in EFSA, 2014a].

Schoenmakers ACM (2003). Histopathology peer review for 90-day oral toxicity study with carvone min 95 % by daily gavage in the rat. Generated by: NOTOX B.V., 's Hertogenbosch, The Netherlands. Company file no. 391062. Makhteshim file TO-1267. GLP, Unpublished, Confidential report [cited in EFSA, 2014a].

Stoner GD, Shimkin MB, Kniazeff AJ, Weisburger JH, Weisburger EK and Gori G (1973). Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Research 33, 3069-3085.

Wanxiang International (Europe) Limited (2015). European Chemicals Agency. REACH registration dossier for l-p-mentha-1(6),8-dien-2-one, CAS RN 6485-40-1. First published 01-May-2013, last modified 24-Dec-2015. https://echa.europa.eu/registration-dossier/-/registered-dossier/12336

# APPENDIX: The (b) (4) database and databank

(b) (4)

(b) (4) includes information from peer-reviewed toxicology and nutrition journals as well as secondary sources and websites. In addition to primary literature on the health effects of chemicals, (b) (4) covers official publications and evaluations issued by authoritative groups including:

- WHO/IPCS reports and evaluations (including CICADs and EHCs, and IARC, JECFA and JMPR monographs), and the WHO Air Quality and Drinking-Water Quality Guidelines
- OECD SIDS dossiers/SIARS
- IUCLID data sets
- EU Risk Assessment Reports
- EU expert committee opinions (including EU scientific committees, and EFSA scientific panels) and other reports from EU agencies and institutes etc (including ECHA, ECVAM, EMA and CPS&Q)
- ECETOC, HERA, Council of Europe and other pan-European programmes
- UK government agency (including Defra, EA, FSA, DoH, HSE, HPA, PSD and VMD) and advisory committee (e.g. COT, COM, COC, ACNFP, SACN, ACP, ACAF, VPC, VRC and ACRE) reports and evaluations
- Opinions from other UK organisations such as the Royal Society
- US agency reports and evaluations (EPA, ATSDR, FDA, NTP, OSHA, NCEA, CFSAN, CERHR, NIEHS, CDC, OEHHA and ACGIH)
- Health Canada evaluations
- BUA, DFG, BG Chemie and BfR reports and monographs
- Gezondheidsraad opinions, including those from its various committees such as DECOS
- RIVM reports
- Danish EPA reviews
- Reports and other information provided by Swedish governmental organisations, including the National Food Administration and the Swedish Chemicals Agency
- Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals
- Australian agency reviews including NICNAS Priority Existing Chemical Assessments, APMVA reports and (jointly with New Zealand) FSANZ assessments
- Japanese Chemical Industry Ecology-Toxicology & Information Center reports
- CIR, RIFM and other specialist industry groups
- (b) (4)