# Overview information for

## Isoprene

# Isoprene (2-methyl-1,3-butadiene)

| | |
|---|---|
| **MAK value (2008)** | **3 ml/m³ ≙ 8.4 mg/m³** |
| **Peak limitation (2008)** | **Peak Limitation Category II, excursion factor 8** |
| **Absorption through the skin** | **–** |
| **Sensitization** | **–** |
| **Carcinogenicity (2008)** | **Category 5** |
| **Prenatal toxicity (2008)** | **Pregnancy Risk Group C** |
| **Germ cell mutagenicity (2008)** | **Category 5** |
| **BAT value** | **–** |
| Synonyms | Hemiterpene |
| | Isopentadiene |
| | β-methylbivinyl |
| | 2-methylbutadiene |
| Chemical name | 2-methylbuta-1,3-diene |
| CAS number | 78-79-5 |
| Formula | $H_2C= C(CH_3)–CH=CH_2$ |
| | $C_5H_8$ |
| Molecular weight | 68.12 g/mol |
| Melting point | –120°C (CambridgeSoft 2006) |
| Boiling point | 34°C (CambridgeSoft 2006) |
| Density at 20°C | 0.681–0.69 g/cm³ (BG Chemie 2000) |
| Vapour pressure at 25°C | 733.3 hPa (SRC 2006) |
| log $K_{ow}$ | 2.42 (SRC 2006) |
| Solubility | 642 mg/l water (SRC 2006) |
| **1 ml/m³ (ppm) ≙ 2.79 mg/m³** | **1 mg/m³ ≙ 0.358 ml/m³ (ppm)** |

The MAK-Collection Part I, MAK Value Documentations 2015
DFG, Deutsche Forschungsgemeinschaft

Evaluations summarizing studies performed on isoprene are available from BG Chemie (BG Chemie 2000) and the OECD High Production Volume Chemicals Programme (OECD 2005).

Isoprene is used in the synthesis of poly(cis-1,4-isoprene) in car tyre production, for the production of styrene/isoprene/styrene block copolymers and butyl rubber (copolymerisate with isobutene), in the production of hydrocarbon resins (petroleum resins), and for the synthesis of terpenes (BG Chemie 2000).

In three plants producing different monomers and elastomers, in which 325 workers were employed, air samples were collected at the workplace for four hours and analysed for isoprene. Isoprene concentrations of up to 1 ml/m$^3$ were found in 398 of 435 samples (91.3%), of 1–5 ml/m$^3$ in 25 samples (5.8%), 5–10 ml/m$^3$ in 6 samples (1.4%), and more than 10 ml/m$^3$ (1.4%) in 6 samples (Leber 2001; Lynch 2001).

## 1 Toxic Effects and Mode of Action

In mice, significantly increased incidences of Harderian gland adenomas occurred after repeated inhalation of isoprene at 70 ml/m$^3$ and above. Hepatocellular adenomas and carcinomas, alveolar/bronchiolar adenomas and carcinomas, adenomas and carcinomas of the forestomach and histiocytic sarcomas occurred at higher concentrations. In rats, isoprene produces significantly increased incidences of mammary gland tumours at 220 ml/m$^3$ and above. Benign tumours of the kidney and testicular interstitial cells occurred at higher concentrations. Isoprene has genotoxic effects in vivo. An increased number of micronuclei-containing erythrocytes in the peripheral blood and an increased SCE frequency in the bone marrow were observed in mice after inhalation exposure.

Isoprene itself and its monoepoxides 1,2-epoxy-2-methyl-3-butene and 1,2-epoxy-3-methyl-3-butene have no genotoxic effects in vitro. The metabolite methyl-1,2:3,4-diepoxybutane, a diepoxide, produces mutations.

Isoprene is endogenously formed in humans. The formation rate in humans is approximately 0.2 μmol/kg body weight and hour; in rats and mice, the endogenous quantity produced is below the detection limit. Using a physiological toxicokinetic model, it was calculated that approximately 90% of the endogenously formed isoprene in humans is transformed to metabolites (not further specified) and approximately 10% is exhaled in unchanged form. Acute toxicity after inhalation exposure is low.

Mice reacted to repeated inhalation with greater sensitivity than rats. Macrocytic anaemia and epithelial hyperplasia of the forestomach occurred at 700 ml/m$^3$ and above in mice. In the males, degeneration of the olfactory epithelium was observed at 7000 ml/m$^3$. However, no substance-specific effects occurred in rats on exposure up to 7000 ml/m$^3$. In this species, there is no indication of sensitization of the skin or of the respiratory tract.

In mice, initial effects on fertility occurred after 13-week inhalation at 700 ml/m$^3$ and above. Thus, in the males, the absolute weight of epididymides and cauda epididymides and sperm motility, sperm concentration, number of spermatids and spermatid heads per testis are reduced. In female mice, the oestrous cycle is prolonged at 7000 ml/m$^3$. In rats, isoprene concentrations of up to 7000 ml/m$^3$ had no effects on fertility. In mice, developmental toxicity such as reduced body weight of foetuses was observed after inhalation at 1400 ml/m$^3$ and above. Maternal toxicity occurred at 7000 ml/m$^3$. In rats, isoprene produces neither developmental nor maternal toxicity after inhalation up to 7000 ml/m$^3$.

## 2 Mechanism of Action

### Formation of haemoglobin adducts

As with butadiene, the monoepoxides of isoprene form adducts with haemoglobin. However, the potency of effects of isoprene is lower by several orders of magnitude (Tareke et al. 1998, see Supplement "1,3-Butadien" 1998, only available in German), as the $S_N2$ type reaction at the C2 atom, which occurs with the butadiene monoepoxides, is suppressed by the methyl group. Preference is given to the $S_N1$ type reaction, for which reason the monoepoxides of isoprene are rapidly hydrolysed. An $S_N2$ type reaction of the isoprene monoepoxides with nitrogen or sulfur nucleophiles at the C3 atom is, however, possible (Bleasdale et al. 1996; Watson et al. 2001).

### Genotoxicity

In an in vitro study, DNA adducts were detected after 24-hour incubation of 2′-desoxyguanosine or single- and double-strand calf thymus DNA with isoprene-1,2-oxide and isoprene-3,4-oxide. N7-(2′-hydroxy-2′-methyl-3′-butene-1′-yl)guanine, N7-(1′-hydroxy-2′-methyl-3′-butene-2′-yl)guanine, N7-(1′-hydroxy-3′-methyl-3′-butene-2′-yl)guanine and N7-(2′-hydroxy-3′-methyl-3′-butene-1′-yl)guanine were formed after deglycosylation (Begemann et al. 2004). The formation of DNA adducts after in vivo exposure to isoprene has to date not been investigated.

One metabolite of isoprene, the diepoxide methyl-1,2:3,4-diepoxybutane, has mutagenic effects (Gervasi et al. 1985). Isoprene itself had no mutagenic effects and nor did it produce increased incidences of SCE and chromosome aberrations (Kushi et al. 1985; de Meester et al. 1981; Mortelmans et al. 1986; NTP 1983, 1995, 1999). The monoepoxides 1,2-epoxy-2-methyl-3-butene and 1,2-epoxy-3-methyl-3-butene also had no mutagenic effects (Gervasi et al. 1985). Accordingly, isoprene itself or its monoepoxides are not responsible for the genotoxic effects, but rather its diepoxides or other metabolites.

**Carcinogenicity**

Already after only 26 weeks of isoprene inhalation increased frequencies of K-ras- and H-ras-mutations in the isoprene-induced tumours of the Harderian glands, the lungs and the forestomach were produced in mice. Here, the frequencies of the following mutations were increased: A → T-transversions in K-ras codon 61 and C → A transversions in H-ras codon 61 (Harderian gland), A → T transversions in K-ras codon 61 (lung) and G → C transversions in K-ras codon 13 and A → T transversions in H-ras codon 61 (forestomach). The authors concluded that the activation of K-ras or H-ras is an important and early step in the formation of these tumours, and that ras mutations and promoting mechanisms contribute to the process of tumour formation (Hong et al. 1997; Sills et al. 1999 a, b, 2001).

# 3 Toxicokinetics and Metabolism

## 3.1 Endogenous formation of isoprene

### 3.1.1 Endogenous formation in humans

Isoprene is formed endogenously in humans, probably from dimethylallyl pyrophosphate (Deneris et al. 1984, 1985), a precursor of cholesterol. The peroxidation of squalene is being discussed as a further source of endogenous isoprene (Stein and Mead 1988). It has also been proposed that isoprene is produced from the degradation of farnesyl- (3 isoprene units) or geranylgeranyl residues (4 isoprene units) of prenylated proteins (Zhang and Casey 1996).

In a series of studies, isoprene was measured in the exhaled air of healthy humans not exposed to exogenous isoprene (Table 1).

As Table 1 shows, the mean isoprene concentrations in the exhaled pulmonary air of populations of awake adults calculated from reference data comprise a concentration range between 11 and 477 µl/m$^3$. With the exception of the data by Stone et al. (1993), the range of the group mean values decreased to between 25 and 119 µl/m$^3$ from 1991 on. This effect can probably be attributed to improved analytical methods. The isoprene concentration in the exhaled air of an individual is greatly variable: it covers a range of half an order of magnitude and is markedly dependent on the intensity of physical activity (Karl et al. 2001; Turner et al. 2006). Physical activity, via changes in cardiac output, influences elimination velocity via exhalation and thus – in the short-term – the concentration of endogenous isoprene in exhaled air. This increases at first rapidly together with the cardiac output before returning to a point lower than the initial value – but with a continued high cardiac output, as the concentration in the blood is reduced due to more rapid exhalation. The endogenous formation rate of isoprene thereby remains unchanged

**Table 1** Isoprene concentrations in the alveolar or pulmonary exhaled air of healthy volunteers – measured concentrations and calculated pulmonary concentrations as comparative parameter

| Number of volunteers | Sex (age in years) | Concentration measured in exhaled air (mean value[a)] ± standard deviation) (range) | | Converted concentration in pulmonary exhaled air (mean value[a)] ± standard deviation) (range) [µl/m³] | References |
|---|---|---|---|---|---|
| 13 | – [9)] | 230 (90–450) | [µl/m³] | 230 | Jansson and Larsson 1969 |
| 25 | 11 ♀, 14 ♂ (adults) | 28.4 ± 12.3 | [nmol/l] | 477 ± 206[1)] | DeMaster and Nagasawa 1978 |
| | | 28.3 ± 7.4 | [nmol/l] | 475 ± 124[1)] | |
| 54 | 19 ♀, 35 ♂ (18–60) | 28.9[4)] | [ng/l] | 11[1), 4)] | Krotoszynski et al. 1979 |
| 50 | 30 ♀, 20 ♂ (15–60) | 14.6 ± 6.4 | [nmol/l] | 367±161[1)] | Cailleux and Allain 1989 |
| 5 | ♂ | 0.99 ± 0.58 | [nmol/l] | 25 ± 15[1)] | Phillips and Greenberg 1991 |
| 12 | (20–30) | 1.62 ± 0.976[6)] | [nmol/l] | 41 ± 25[1)] | Kohlmüller and Kochen 1993 |
| 5 | 5 ♂ (18–50) | 21.7 ± 6.4 | [nmol/l] | 364 ± 107[1)] | Stone et al. 1993 |
| 43 | 23 ♀, 20 ♂ (22–75) | 7.05 ± 3.53 | [nmol/l] | 118 ± 59[1)] | Mendis et al. 1994 |
| 15 | –[9)] | 7.1 ± 1.0 | [nmol/l] | 119 ± 17[1)] | Mendis et al. 1995 |
| ≥ 40 | –[9)] | 250 (70–580) | [µl/m³] | –[3)] | Hansel et al. 1995 |
| 16 | 10 ♀, 6 ♂ (adults) | 3.89 ± 2.43 | [nmol/l] | 98 ± 61[1)] | Jones et al. 1995 |
| | | 3.46 ± 0.84 | [nmol/l] | 87 ± 21[1)] | |
| 10 | 9♂, 1 ♀ (adults) | (0.37–3.2) | [nmol/l] | (9–80)[1)] | Foster et al. 1996 |
| 4 | 1 ♀, 3♂ (19–34) | 4.7 | [nmol/l] | 117 ± 11[1), 5)] | Filser et al. 1996; Csanády and Filser 2001 b |
| 141 | (22–74) | 240 ±120 | [µl/m³] | –[3)] | Taucher et al. 1997 |

**Table 1** (Continued)

| Number of volunteers | Sex (age in years) | Concentration measured in exhaled air (mean value[a)] ± standard deviation) (range) | | Converted concentration in pulmonary exhaled air (mean value[a)] ± standard deviation) (range) [µl/m³] | References |
|---|---|---|---|---|---|
| 1 | –[9)] | 10 ± 1.4 | [nmol/l] | 168 ± 23[1)] | Grote and Pawliszyn 1997 |
| 10 | –[9)] | (30–135) | [µl/m³] | –[3)] | Fenske and Paulson 1999 |
| 6 | 1 ♀, 5 ♂ (24–60) | 95, 58, 54, 113, 92, 48 | [µl/m³] | 51 ± 18[2), 6)] | Smith et al. 1999 |
| 29 | ♀ and ♂ (25–65) | 83 ± 45 | [µl/m³] | 55 ± 30[2)] | Španělet al. 1999 |
| 1 | –[9)] | 3 (2–4) | [nmol/l] | 75[1)] | Hyšpler et al. 2000 |
| 10 | 2 ♀, 8 ♂ (22–59) | 1.7 (0.5–2.5) | [nmol/l] | 43[1)] | Mitsui et al. 2000 |
| 8 | 3 ♀, 5 ♂ (15–30) | (55–185) | [µl/m³] | (55–185) | Senthilmohan et al. 2000 |
| 17 | 9 ♀, 8♂ (24–62) | 89 ± 36 | [µl/m³] | 59 ± 24[2)] | Davies et al. 2001 |
| 8 | (adults, at rest) | 157 ± 67 | [µl/m³] | –[3)] | Karl et al. 2001 |
| 8 | (adults, physically active) | 385 ±140 | [µl/m³] | –[3)] | Karl et al. 2001 |
| 31 | 10 ♀, 21 ♂ (30–46) | 1.12 ± 0.14[7)] | [nmol/l] | 28 ± 20[1), 6)] | McGrath et al. 2001 |
| 4 | 2 ♀, 2 ♂ (28–39) | 2.4 ± 0.90 | [nmol/l] | 40 ± 15[1)] | Lärstad et al. 2002 |
| 10 | 4 ♀, 6 ♂ (28–41) | 5.99 | [nmol/l] | 151[1)] | Scholpp et al. 2002 |
| 5 | 2 ♀, 3 ♂ (27–65) | 82 ± 42[6)] (55–121) | [µl/m³] | –[3)] | Diskin et al. 2003 |
| 16 | 8 ♀, 8 ♂ (adults) | 6.07 ± 1.75 | [nmol/l] | l02 ± 29[1)] | Cope et al. 2004 |
| 50 | 23 ♀, 27 ♂ (55.7[8)]) | 3.8 ± 3.9[6)] | [nmol/l] | 64 ± 65[1)] | Poli et al. 2005 |

**Table 1** (Continued)

| Number of volunteers | Sex (age in years) | Concentration measured in exhaled air (mean value[a)] ± standard deviation) (range) | | Converted concentration in pulmonary exhaled air (mean value[a)] ± standard deviation) (range) [µl/m³] | References |
|---|---|---|---|---|---|
| 15 | 5 ♀, 10 ♂ (adults) | 3.91[8)] | [nmol/l] | 98[1), 8)] | Statheropoulos et al. 2005 |
| 20 | 11 ♀, 9 ♂ (8–29) | 114 | [µl/m³] | 76[2)] | Barker et al. 2006 |
| 66 | 66 ♀ (19–79) | 57.5 ± 27.8 | [µl/m³] | 38 ± 19[2)] | Lechner et al. 2006 |
| 60 | 60 ♂ (19–79) | 80.6 ± 34.1 | [µl/m³] | 54 ± 23[2)] | |
| 30 | 11 ♀, 19 ♂ (24–59) | 122 ± 63[6)] | [µl/m³] | 81 ± 42[2)] | Turner et al. 2006 |
| 14 | 11 ♀, 3 ♂ (24–64) | 125[8)] | [µl/m³] | 83[2), 8)] | Lärstad et al. 2007 |

[a)] unless otherwise stated;
[1)] conversion of nmol/l to µl/m³ in pulmonary exhaled air using the factors 16.8 (measurements in alveolar air) and 25.1 (measured in pulmonary exhaled air); conversion of ng/l in pulmonary exhaled air to µl/m³ by multiplication with 0.369;
[2)] conversion of alveolar to pulmonary concentration by multiplication with $^2/_3$ (according to Fiserova-Bergerova 1983);
[3)] not determinable from the method described, whether alveolar or pulmonary exhaled air;
[4)] geometric mean value;
[5)] standard deviation given by authors;
[6)] standard deviation calculated from given values or parameters;
[7)] standard error;
[8)] median;
[9)] no data given

(Karl et al. 2001). During the night (no data given as to whether volunteers were awake or just awakened), markedly higher isoprene concentrations were measured in the exhaled air (data not included in Table 1) than during the day (DeMaster and Nagasawa 1978; Stone et al. 1993). Cailleux and Allain (1989) found no increase in isoprene concentration in persons awake during the night, but only in volunteers awakened shortly before measurement. Taucher et al. (1997) also found increased isoprene concentrations in the exhaled air of persons after wakening or in the resting state. Increased nocturnal isoprene exhalation is explained as being a circadian rhythm (DeMaster and Nagasawa 1978; Stone et al. 1993) and with a nocturnal increase in cholesterol synthesis (Salerno-Kennedy and Cashman 2005). According to Karl et al. (2001) the increase in isoprene concentration in the exhaled air can, however, possibly be attributed to the increase in heart rate alone, produced by waken-

ing a sleeping volunteer and his/her subsequently getting up. There was a great inter-individual variation in the isoprene concentration in the exhaled air. In 29 of 30 volunteers, whose alveolar air isoprene concentrations were measured at rest on a weekly basis for half a year, the individual mean concentrations were between 38 and 308 µl/m$^3$. In one volunteer, the mean isoprene concentration was only 5 µl/m$^3$ (mean value and standard deviation across all 30 study participants; their age range is given in Table 1; Turner et al. 2006). No influence of mental stress, age, body fat (determined as body mass index) or sex on exhalation data could be found.

DeMaster and Nagasawa (1978) also found no significant age- or sex-related differences in alveolar isoprene concentration in a study with 66 women and 60 men. Lechner et al. (2006) obtained results showing that the mean exhaled isoprene concentrations in women are somewhat lower than in men. In children also, the concentrations were lower than in adults (Taucher et al. 1997). No or very little exhaled isoprene was found in the newborn (Nelson et al. 1998); in children of pre-school age, lower isoprene concentrations in the exhaled air were found than in school children (Nelson et al. 1998), and the values were also lower in adults between 19 and 29 years than in adults between 30 and 79 years (Lechner et al. 2006). The causes for these differences are not clear. In the study by Nelson et al. (1998), a linear relationship was derived between the isoprene concentration and the ages of the children or adolescents, whereby the regression coefficient was very small at $r^2 = 0.297$. The observed age-dependence of the breath isoprene concentration is perhaps only due to various age-specific physical activities. A relationship between isoprene concentration and cholesterol metabolism was demonstrated in three studies: a decrease in isoprene concentration in the exhaled air was found after medication for hypercholesteraemia using inhibitors of 3-hydroxy-3-methylglutaryl-CoA-reductase (HMG-CoA-reductase) and after administration of an inhibitor of HMG-CoA-reductase to healthy volunteers (Karl et al. 2001; Stone et al. 1993; Zadak et al. 1999). HMG-CoA-reductase is the rate-limiting enzyme in cholesterol biosynthesis. It catalyses the formation of mevalonic acid, from which isoprene is formed non-enzymatically by rat liver cytosol via dimethylallyl pyrophosphate, an intermediate product (Deneris et al. 1984, 1985). The finding of reduced isoprene exhalation after administration of HMG-CoA-reductase inhibitors thus supports the theory put forward by Deneris et al. (1984, 1985), according to which endogenous isoprene is a natural by-product of cholesterol synthesis. The fact that a parallel decrease in cholesterol synthesis and exhaled isoprene was found in 8 male volunteers after six weeks of a cholesterol-rich diet agrees with this hypothesis (Stone et al. 1993). Using gas chromatography and flame ionization detection (GC/FID), Cailleux et al. (1992) found isoprene concentrations of 37 ± 25 nmol/l (mean value ± standard deviation; range: 15–70 nmol/l) in the blood of 10 volunteers of both sexes. In a more recent study, mean isoprene concentrations of 10.29 ± 6.17 nmol/l (median: 9.08 nmol/l; range: 0.52–24.5 nmol/l) were measured using gas chromatography and mass spectrometry (GC/MS) in the venous blood and of 6.68 ± 4.71 nmol/l (median: 5.73 nmol/l; range: 0–18.8 nmol/l) in the arterial blood of

33 mechanically ventilated patients (Miekisch et al. 2001). In a further study (Statheropoulos et al. 2005), endogenous isoprene was also found in the blood of volunteers. A mean endogenous isoprene concentration of 5.2 ± 4.0 nmol/l in the venous blood was obtained in a model calculation based on more recent measurements of isoprene in the exhaled air of 337 adults of both sexes (see Section 3.6).

### 3.1.2 Endogenous formation in animals

Gelmont et al. (1981) found endogenous isoprene in the exhaled air of suckling rats. Several days after weaning no more isoprene was found. No exhaled isoprene was found in mice, guinea pigs, chickens, rabbits, dogs and pigeons (Gelmont et al. 1981; DeMaster and Nagasawa 1978). On repetition of equivalent exposures, the exhalation of endogenous isoprene in rats and mice reported by Peter et al. (1987, 1990) was later found to be probably erroneous. In this case it was found that a column filling material incapable of separating endogenous isoprene from endogenous acetone was used in flame ionization gas chromatography (Filser et al. 1996). In the blood of rats, rabbits, ponies, dogs, cows and sheep, however, very low isoprene concentrations (< 1 nmol/l) were determined using mass selection gas chromatography. They were less than one thirtieth of the concentrations measured in the blood of volunteers (Cailleux et al. 1992). Concentrations ranging from 0.2 up to 1.3 nmol/l in the venous and from 0 up to 0.8 nmol/l in the arterial blood were obtained for endogenous isoprene in mechanically ventilated pigs. The isoprene concentrations were between 0.3 and 0.7 nmol/l in the venous blood of rabbits (detection limit: 0.05 nmol/l; Miekisch et al. 2001).

## 3.2 Absorption, distribution, elimination

Groups of male F344 rats inhaled (nose-only) $^{14}$C-isoprene concentrations of 0, 8, 260, 1480 or 8200 ml/m$^3$ up to six hours. The $^{14}$C levels in urine and faeces, the exhaled substances and the $^{14}$C content remaining in the organism were determined in four animals per group during the following 66 hours. Five rats per group were exposed in special plethysmograph tubes and their pulmonary ventilation determined during exposure lasting six hours. In addition, the inhaled amount of $^{14}$C-isoprene and the retention of $^{14}$C-isoprene were estimated through comparison with the $^{14}$C still present in the organism at the end of exposure. This was 19% at 8 ml/m$^3$, 9.1% at 260 ml/m$^3$, 5.8% at 1480 ml/m$^3$ and 4.5% at 8200 ml/m$^3$. In the same way, the metabolized portion of the inhaled $^{14}$C-isoprene was estimated by comparing the calculated quantities of inhaled $^{14}$C-isoprene with the $^{14}$C quantities retrieved in urine, faeces, total organism and exhaled $^{14}CO_2$ up to 66 hours after end of exposure. This was 25.3% at 8 ml/m$^3$, 12.0% at 260 ml/m$^3$, 4.7% at 1480 ml/m$^3$

and 3.6% at 8200 ml/m$^3$. At all exposure concentrations more than 75% of the quantity attributed to the $^{14}$C metabolism was found in urine. The mean half-life of $^{14}$C in urine was 10.2 hours, independent of the exposure concentration. Furthermore, the $^{14}$C activity in relation to exposure concentration and duration was measured in the blood, and at 1480 ml/m$^3$ also in the nose, lungs, liver, kidney and fat. The highest $^{14}$C quantities were found in the fat after six hours. The authors attributed the $^{14}$C-activities in the blood and the tissues to $^{14}$C-isoprene itself as well as specific metabolites (e.g. diol, epoxide and diepoxide) (Dahl et al. 1987, 1990).

Male B6C3F$_1$ mice were exposed (nose-only) by inhalation to $^{14}$C-labelled or non-labelled isoprene at concentrations of 0, 20, 200 or 2000 ml/m$^3$ for up to six hours in special plethysmograph tubes. Steady state was reached at all exposure concentrations in the blood within 15 to 30 minutes after start of exposure. The corresponding mean isoprene concentrations in the blood determined by gas chromatography were 24.8 ng/ml (20 ml/m$^3$), 830 ng/ml (200 ml/m$^3$) or 6800 ng/ml (2000 ml/m$^3$). The $^{14}$C retained in the organism, calculated at the end of exposure in analogy with Dahl et al. (1987), was 5.9% at 18 ml/m$^3$, 8.9% at 205 ml/m$^3$ and 3.8% at 2000 ml/m$^3$, related to the inhaled quantity of $^{14}$C-isoprene. Between 52% (at 18 ml $^{14}$C-isoprene/m$^3$) and 73% (at 2000 ml $^{14}$C-isoprene/m$^3$) of the metabolite-associated radioactivity was excreted in the urine over a 64-hour post-exposure period. The amounts of inhaled $^{14}$C-isoprene in the mice identified as metabolites in this post-exposure period were less than in the rats (see Dahl et al. 1987, 1990). At 18, 205 and 2000 ml/m$^3$ they amounted to 4.6%, 7.5% and 2.3% (Bond et al. 1991).

Groups of three male F344 rats and four male B6C3F$_1$ mice received single intraperitoneal injections of $^{14}$C-isoprene in doses of 64 mg/kg body weight in corn oil. Of this, about 50% unchanged $^{14}$C-isoprene was exhaled; about 32% was excreted in the urine in the form of metabolites. Recovery was about 91% in both species. In rats, 2-hydroxy-2-methyl-3-butenoic acid (53%) as well as 2-methyl-3-buten-1,2-diol (23%) and the C-1 glucuronide of 2-methyl-3-buten-1,2-diol (13%) were found in the urine. These metabolites were identified and quantified by NMR spectroscopy and gas chromatography with mass selective detection. Numerous other isoprene metabolites were additionally found in the urine of mice. The percentage of radioactivity in the urine of mice associated with an unidentified polar fraction was comparatively higher than in that of rats (mouse 25%, rat 7%) (Buckley et al. 1999).

No studies are available on dermal absorption. Starting with a water solubility of 642 mg/1 and a log $K_{ow}$ of 2.42, dermal fluxes of 0.023 or 0.026 mg/cm$^2$ and hour are obtained for a saturated aqueous isoprene solution using the models of Guy and Potts (1993) and Wilschut et al. (1995). This would correspond to a total dermal absorption of 46.9 or 52.6 mg isoprene after exposure of both hands and lower arms (about 2000 cm$^2$) for one hour.

Studies with other hydrocarbons show that dermal absorption from the gas phase is low compared with the uptake from inhalation (McDougal et al. 1990).

### 3.3 Metabolism

The metabolism of isoprene to epoxides and diols (according to Chiappe et al. 2000) is shown schematically in Figure 1. In the presence of NADPH and liver microsomes of different test species, isoprene (1) is metabolized by oxidation forming the two epoxides 1,2-epoxy-2-methyl-3-butene (2) and 1,2-epoxy-3-methyl-3-butene (3) (Chiappe et al. 2000; Del Monte et al. 1985; Longo et al. 1985; Wistuba et al. 1994). The 1,2-epoxy-2-methyl-3-butene (2) formed proportionally to a considerably greater extent is subject to rapid, mainly non-enzymatic hydrolysis to form 1,2-dihydroxy-2-methyl-3-butene (4). In contrast, 1,2-epoxy-3-methyl-3-butene (3) is hydrolysed more slowly to form 1,2-dihydroxy-3-methyl-3-butene (5), mainly catalysed by the microsomal epoxide hydrolase (Chiappe et al. 2000). 1,2-Epoxy 3-methyl-3-butene (3) is also the main initial product for microsomal oxidation to 1l,2:3,4-diepoxy-2-methyl-butane (6) (Chiappe et al. 2000; Del Monte et al. 1985; Longo et al. 1985) which, however, can also be formed from the other mono-

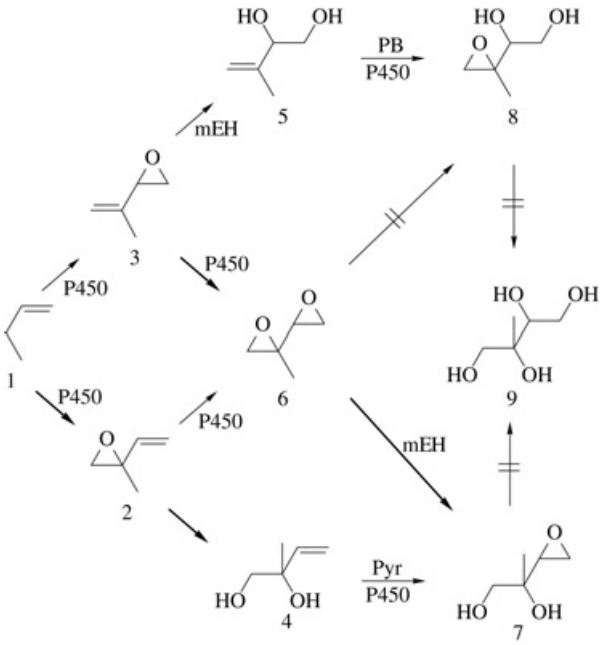



**Figure 1** Metabolism of isoprene in microsomes from rat liver (according to Chiappe et al. 2000)
P450: cytochrome P450-dependent monooxigenases;
mEH: microsomal epoxide hydrolase,
PB: phenobarbital pretreatment,
Pyr: pyrazole pretreatment

epoxide (2) (Chiappe et al. 2000; Wistuba et al. 1994). Catalysed by the microsomal epoxide hydrolase 1,2:3,4-diepoxy-2-methyl butane (6) is hydrolysed to 1,2-dihydroxy-2-methyl-3,4-epoxy butane (7). The formation of 1,2-dihydroxy-2-methyl-3,4-epoxybutane (7) from 1,2-dihydroxy-2-methyl-3-butene (4) or of 1,2-dihydroxy-3-methyl-3,4-epoxybutane (8) from 1,2-dihydroxy-3-methyl-3-butene (5) could only be demonstrated after pretreatment of rats with CYP450-inducing substances (pyrazole or phenobarbital). It was not possible to hydrolyse either of these epoxy diols by microsomal epoxide hydrolase to form 1,2,3,4-tetrahydroxy-2-methylbutane (9) (Chiappe et al. 2000). All epoxides and diols are present in the form of optical isomers (Chiappe et al. 2000; Golding et al. 2003).

The oxidation of isoprene to the monoepoxides in vitro was mainly produced by CYP2E1, followed by CYP2B6 (Bogaards et al. 1996). Inhibition of epoxide hydrolase by cyclohexene oxide in the liver microsomes of humans, mice and rats resulted in similar rates of monoepoxide formation. Without inhibition of the enzyme, the total amount of monoepoxides was twice as high for mouse liver microsomes than for rat and even 15 times as high as for human liver microsomes. In the authors' opinion, the differences in epoxide hydrolase activity between species are responsible for the varying toxicity of isoprene (Bogaards et al. 1996). CYP2E1 was the only cytochrome P450 isoenzyme showing detectable formation of the diepoxide. Both monoepoxides were oxidized by CYP2E1 to the diepoxide at similar rates. The enzymatic activities were 780 (substrate: 3.2-epoxy-2-methyl-3-butene) or 666 (1,2-epoxy-3-methyl-3-butene) pmol/min and nmol cytochrome P450 in humans, 1210 or 886 pmol/min and nmol cytochrome P450 in CD1 mice, 806 or 967 pmol/min and nmol cytochrome P450 in $B6C3F_1$ mice and 1150 or 1360 pmol/min and nmol cytochrome P450 in Wistar rats, respectively (Bogaards et al. 1996). Compared with mice and rats, the activity of GSH transferases in the microsomal fraction from the human liver was lower by a factor of 25 to 50 (Bogaards et al. 1999). The half-life of 1,2-epoxy-2-methyl-3-butene and 1,2-epoxy-3-methyl-3-butene in buffer solution was 1.25 and 73 hours, respectively (37°C, pH 7.4) (Gervasi et al. 1985).

A number of studies on enantioselectivity and stereoselectivity have been performed (Chiappe et al. 2000; Small et al. 1997; Wistuba et al. 1994). Microsomes from the liver of male rats showed a marked preference for the formation of the (S)-enantiomers of monoepoxides (Small et al. 1997; Wistuba et al. 1994). In contrast, liver microsomes from men, female dogs or male monkeys preferentially formed the (R)-enantiomer. No enantioselectivity was found in liver microsomes of women, male mice, female rabbits and female rats (Small et al. 1997). (S)-enantiomers were preferably transformed by epoxide hydrolase (in rat and mouse). (R)-1,2-epoxy-3-methyl-3-butene was hydrolysed more rapidly by mouse liver microsomes than by rat liver microsomes (Wistuba et al. 1994).

### 3.4 Haemoglobin adduct formation

Groups of three male Sprague Dawley rats and three male B6C3F$_1$ mice were given intraperitoneal injections of $^{14}$C-isoprene in single doses of 0.3, 3, 300, 1000 or 3000 µmol/kg body weight (about 0.02, 0.2, 20.4, 68.1 or 204.4 mg/kg body weight) or daily doses of 500 µmol/kg body weight (about 34 mg/kg body weight and day) in corn oil for 1, 2 or 3 days. Twenty-four hours after the last injection, the formation of radioactively labelled haemoglobin adducts was linear up to 34 mg/kg body weight and day in both species. About 40 pmol haemoglobin adduct per mg globin was found in both species at the highest administered dose of 204.4 mg/kg body weight. $^{14}$C adduct at 0.158 ± 0.035 pmol/mg globin was formed by mice and 0.079 ± 0.016 pmol/mg globin by rats in relation to the retained isoprene/kg body weight. Repeated administration of $^{14}$C-isoprene at 34 mg/kg body weight produced an accumulation of $^{14}$C-haemoglobin adducts (Sun et al. 1989).

Groups of four male B6C3F$_1$ mice were exposed for 6 hours to $^{14}$C-isoprene concentrations of 20, 200 or 2000 ml/m$^3$. Twenty-four hours thereafter, the corresponding haemoglobin adduct levels were 11 ± 0.5, 90 ± 13 and 170 ± 13 pmol/mg globin. In the range of 200 to 2000 ml/m$^3$, retention was not linearly related to exposure concentration, and the formation of Hb adducts not linearly dependent on the retained quantity of isoprene. According to the authors, the considerably higher yield of haemoglobin adducts compared with intraperitoneal administration (Sun et al. 1989) in relation to the absorbed amount of isoprene, must be attributed to the fact that pathways for metabolism of isoprene were saturated by the high systemic concentration after the intraperitoneal bolus (Bond et al. 1991).

By intraperitoneal injection, one male Sprague Dawley rat received 0 or 250 µmol/kg body weight (0, 17 mg/kg body weight) and two male C57/black mice 0, 113 or 227 µmol/kg body weight (0, 7.7, 15.4 mg/kg body weight) in corn oil. In the control animals, the adduct levels were below the detection limit (0.20 pmol/g globin). Two adducts were found in the treated animals, derived from the two monoepoxides 1,2-epoxy-2-methyl-2-butene (A) and 1,2-epoxy-3-methyl-3-butene (B). Per g globin, 0.86 pmol adduct A and 0.43 pmol adduct B was found in the rat. In the treated mice, the adduct levels per g globin were 14 pmol (adduct A) or 0.86 pmol (adduct B) for the 113 µmol/kg group and 28 pmol (adduct A) or 1.5 pmol (adduct B) for the 227 µmol/kg group, respectively. This means that the mice formed approximately 20 times more adducts than the rats (Tareke et al. 1998).

### 3.5 Physiological toxicokinetic models

To obtain a quantitative description of the effects of isoprene exposure in the blood and various tissues of mice, rats and humans resulting from different inhalation exposures, a physiological toxicokinetic model (PT model) was developed (Csanády and Filser 2001 b; Filser et al. 1996). The model (Figure 2) comprises

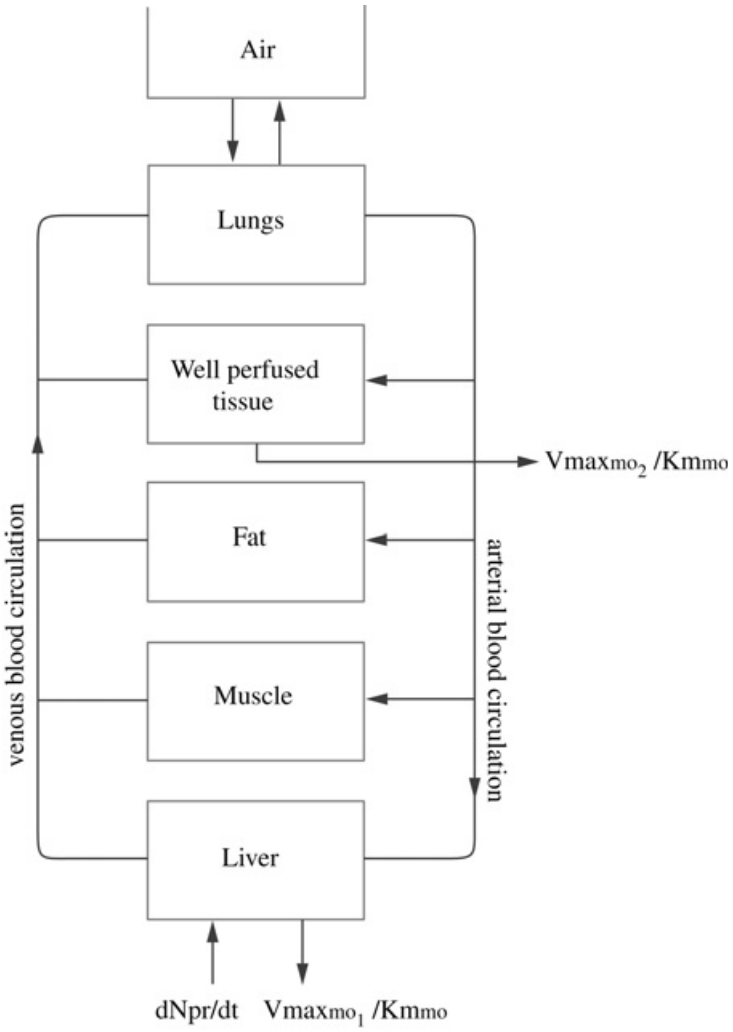



**Figure 2** Physiological toxicokinetic model for inhaled and endogenously formed isoprene. Endogenous formation occurs in the liver. Ninety percent of the isoprene is metabolized in the liver and 10% extrahepatically (Csanády and Filser 2001 b; Filser et al. 1996).

Abbreviations: dNpr/dt, endogenous formation rate;
Vmaxmo1, Vmaxmo2, maximum metabolic rate in liver or well perfused tissue;
Kmmo, apparent Michaelis constant in the venous blood of the liver and well perfused tissue

five compartments representing the lungs, the total of all well-perfused organs and tissues, fat, muscles and liver. The individual organ and tissue compartments are connected to each other by blood circulation.

Inhaled isoprene passes into the arterial blood circulation via the lungs and is distributed together with it throughout the different organ and tissue compartments. Metabolic elimination of the isoprene occurs 90% in the liver and 10% in the well-perfused organ group. The isoprene leaving the compartments is passed with the venous blood circulation back to the lungs, from where it is exhaled according to respiratory activity and its blood:air partition coefficient. As the exhalation of endogenous isoprene could be determined experimentally in four volunteers (3 ♂, 1 ♀), the endogenous formation of isoprene in humans was included into this model. It was incorporated in the liver compartment, in agreement with the results obtained by Gelmont et al. (1981) and Deneris et al. (1985), which had shown that isoprene was formed in the liver. The partition coefficients required for the PT model were experimentally determined at 37°C in vitro. The blood:air partition coefficients measured for mice, rats and humans were 2.04, 2.33 and 0.75. A similar value of 1.87 had been determined by Gargas et al. (1989) in the blood of rats. The PT model was validated by measured concentration-time curves of inhaled or exhaled isoprene in the atmosphere of closed exposure systems (data for humans: Filser et al. 1996; for mouse and rat: Csanády and Filser 2001 b; Peter et al. 1987). Saturation kinetics were determined in the rodent species. The maximum metabolic rates were 410 µmol/h and kg body weight in the mouse and 110 µmol/h and kg body weight in the rat (Csanády and Filser 2001 b). In the rat they were reached at approximately 1500 ml/m$^3$ and not until beyond this value in the mouse.

The half-maximum metabolic rate was reached at 360 ml/m$^3$ in the mouse and at 180 ml/m$^3$ in the rat. In the low concentration range, for which the model predictions in volunteers were experimentally checked at isoprene concentrations ≤ 50 ml/m$^3$, the metabolic rate was directly proportional to the isoprene concentration in the three species. As shown by Csanády and Filser (2001 a), the metabolism of isoprene (represented by alveolar retention at steady state) is limited in this concentration range by the blood circulation through the metabolizing organs. As the absorption and distribution of isoprene do not depend on biochemical but only on physicochemical and physiological factors, it follows that this also applies for the metabolism of the three species in the low exposure range. From the PT model, it was found that the rate of metabolism per kg body weight for inhaled isoprene in the concentration range < 50 ml/m$^3$ was about 8 times faster in the rat (body weight 250 g) and 14 times faster in the mouse (body weight 25 g) than in humans (body weight 70 kg). With isoprene, a metabolic rate of 2.5 µmol/h and kg body weight after exposure to 50 ml/m$^3$ was obtained in humans. As the blood:air partition coefficient of isoprene in both rodent species is markedly higher than in humans, higher isoprene concentrations in the venous blood of mouse and rat were calculated for the same exposure concentrations of isoprene vapour in the atmosphere than in the blood of humans. With exposure to 50 ml/m$^3$, isoprene concentrations of 1.5 µmol/l blood for the mouse, 1.7 µmol/l for the rat and 0.65 µmol/l for humans were obtained with the model simulation for steady state. From the concentration-time curves of endogenously formed, exhaled isoprene measured in the four volunteers using a closed exposure system, an endogenous isoprene formation rate of

23.8 µmol/h was calculated with the PT model for a human weighing 70 kg. Approximately 90% are metabolized and only approximately 10% exhaled. From this, an endogenous isoprene burden of 9.5 nmol/l venous blood is obtained. Using the exhalation data of 337 volunteers as reference, an endogenous isoprene burden of 5.2 ± 4.0 nmol/l blood was obtained with this model (see Section 3.6.). The accuracy of the model predictions was additionally demonstrated by Csanády and Filser (2001 b) using isoprene concentrations measured in the blood of exposed B6C3$F_1$ mice (Bond et al. 1991), of the quantity metabolized by rats after inhalation (Dahl et al. 1987) or exhaled after intraperitoneal administration (Buckley et al. 1999), and the exhalation of endogenous isoprene in humans (Conkle et al. 1975; Gelmont et al. 1981; Jones et al. 1995; Mendis et al. 1994; Mitsui et al. 2000). It was calculated that an adult exhales 3.4 mg endogenous isoprene within 24 hours. Conkle et al. (1975) and Gelmont et al. (1981) published measured isoprene exhalations of 0.36–9.36 mg/24 hours or 2–4 mg/24 hours. The model simulations confirm that the PT model is suitable for the calculation of isoprene burden resulting from endogenous or exogenous exposure to this substance. A further physiological toxicokinetic model is based on in vitro studies on the CYP450-catalysed formation of epoxides, on their hydrolysis by epoxide hydrolase and on the glutathione S-transferase catalysed glutathione conjugation of monoepoxides. Using this model, the concentrations of 1,2:3,4-diepoxy-2-methylbutane in liver and lung resulting from isoprene exposure were predicted for mice, rats and humans (Bogaards et al. 2001). Whereas the simulations showed similar diepoxide concentrations in the two rodent species having different sensitivities to isoprene toxicity, markedly lower levels were obtained in the humans. The model was, however, only validated with isoprene concentrations measured in the blood of mice obtained by Bond et al. (1991). No comparison with available data from rats or humans was undertaken.

## 3.6 Derivation of a MAK value for isoprene

Genotoxic epoxides are formed during the biotransformation of isoprene. It is assumed that the tumorigenic effects observed in long-term studies are attributable to the exposure to these metabolites, mainly to 1,2:3,4-diepoxy-2-methylbutane. The internal exposure to these metabolites is not quantifiable up to now. As exposure parameter, however, the area under the concentration/time curve in the blood (AUC) can be used for its metabolic precursor, isoprene. Isoprene is formed endogenously in humans. The resulting endogenous isoprene exposure can be calculated from the isoprene concentration measured in the exhaled air using a physiological toxicokinetic model validated with experimental human data (Csanády and Filser 2001 b; Filser et al. 1996), and compared with that resulting from an exogenous isoprene exposure. For this reason, the MAK value for exogenous isoprene refers to the AUC obtained with lifelong (80 years) endogenous isoprene formation. The MAK value is established so that exposure under MAK value conditions produces

an additional AUC equal to the AUC from the standard deviation of endogenous isoprene concentration. The MAK value for ethanol was already derived according to the same principle. Ethanol is also formed endogenously and transformed into carcinogenic metabolites.

### Determination of internal exposure to endogenous isoprene

In order to estimate the endogenous isoprene exposure and its standard deviation for the general population from measurements of isoprene concentrations in the exhaled air using the physiological toxicokinetic model, the measured data of as many individuals as possible taken from the results published in Table 1 were used. For calculation, the publications were selected according to the following criteria:

- number of persons, group mean value and standard deviation are available
- at most one study per working group (as recent as possible; number of volunteers as large as possible); this is to keep the bias of method as low as possible, and to avoid the same volunteers repeatedly entering the calculation.

This leaves the publications remaining in Figure 3.

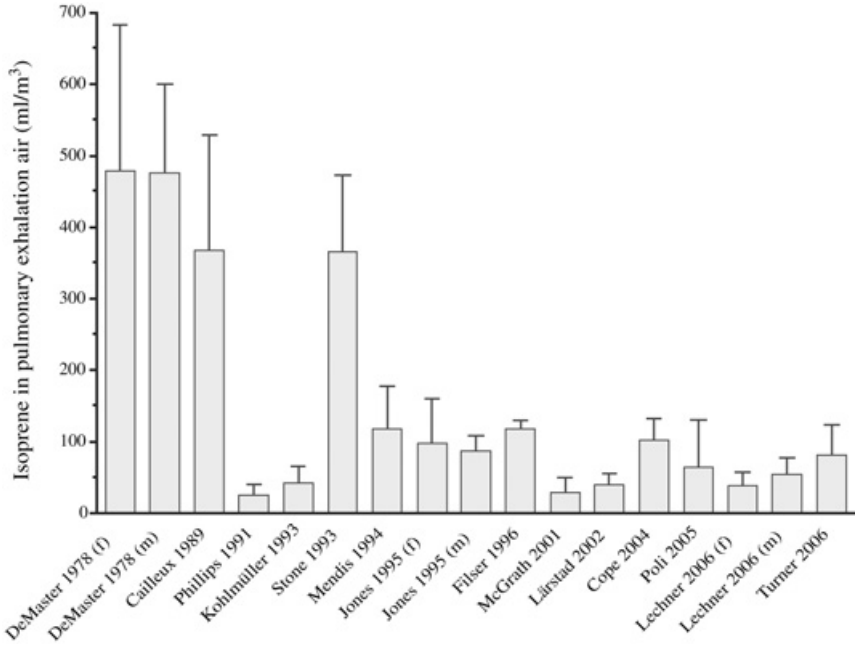



**Figure 3** Isoprene concentrations in the pulmonary exhaled air of healthy volunteers (mean values ± standard deviation); m = male, f = female volunteers; see text for selected literature; each citation with first author only and year of the publications given in Table 1.

Statistically, the exhalation concentrations obtained by DeMaster and Nagasawa (1978), Cailleux and Allain (1989) and Stone et al. (1993) are significantly (ANOVA, post-hoc test according to Bonferroni, $p < 0.05$) higher than those of all other authors, whose data – with the exception of Stone et al. (1993) – were all published after 1990 and obtained using more recent and hence more reliable analytical methods. This is why the data of DeMaster and Nagasawa (1978), Cailleux and Allain (1989) and Stone et al. (1993) are not used in determining the average isoprene concentration and its standard deviation. The exhalation data of all other studies cited in Figure 3 were summarized. From this, a weighted mean concentration in the pulmonary exhaled air of 0.064 ml/m$^3$ and a weighted standard deviation of 0.049 ml/m$^3$ after Sachs (1997) was calculated for the 337 volunteers considered.

The following values (mean value ± standard deviation) are obtained after using these exhalation concentrations in the PT model for adults weighing 70 kg:

| | |
|---|---|
| Endogenous isoprene: | |
| Rate of formation | 13.1 ± 10.0 µmol/h |
| Concentration in venous blood | 5.2 ± 4.0 nmol/l |
| AUC (0–80 years) | 3.6 ± 2.8 mmol × h/l |

Occupational exposure to 10 ml isoprene/m$^3$ (8 hours/day, 5 days/week, 48 weeks/year, 40 years):

| | |
|---|---|
| Additional AUC (40 years) | 9.8 mmol × h/l |
| Expected concentration in venous blood after 8 hours | 133 nmol/l |

If the sum of the AUCs from the mean endogenous and exogenous exposure is to equal the sum of the AUCs of the mean endogenous exposure and its standard deviation (analogous to the procedure used in the MAK value finding for ethanol), an exogenous isoprene concentration of 2.9 ml/m$^3$ in the ambient air is obtained.

The AUC after an 8-hour daily exposure to about 3 ml/m$^3$ lasting 40 years is accordingly of the same magnitude as the AUC with lifelong exposure at the level of the standard deviation of the mean endogenous isoprene concentration.

# 4 Effects in humans

## 4.1 Single exposures

Studies with volunteers to determine acute effects are given in Table 2. One woman and two men inhaled isoprene at 278–27 800 mg/m$^3$ (about 100–10 000 ml/m$^3$) for 5 minutes. Isoprene concentrations of 278 mg/m$^3$ (about 100 ml/m$^3$) were at the

**Table 2** Acute effects of isoprene in volunteers

| Collective | Duration, administration route, concentration, purity | Remarks | Results | References |
|---|---|---|---|---|
| 1 ♀ and 2 ♂ | 5 min; inhalation; 278–27 800 mg/m³ (about 100–10 000 ml/m³); no further details | no data on analytics | 278 mg/m³ (about 100 ml/m³): odour perceptible; 13 900 mg/m³ (about 5000 ml/m³): headache; 27 800 mg/m³ (10 000 ml/m³): marked bronchial irritation (no further details) | BG Chemie 2000 |
| 10 volunteers; no further details | no data on duration; inhalation; 160 mg/m³ (about 57 ml/m³); no further details | no data on analytics | mild irritation of mucous membranes in nose, larynx and pharynx; odour threshold: 10 mg/m³ (about 3.6 ml/m³) (no further details) | Gostinskii 1965 |

limit of odour perception, 695 mg/m³ (about 250 ml/m³) clearly perceptible and 2780 mg/m³ (about 1000 ml/m³) very perceptible. In addition, headache and pronounced headache occurred at 13 900 mg/m³ (about 5000 ml/m³) and 27 800 mg/m³ (about 10 000 ml/m³), respectively. Furthermore, at the highest concentration there was a marked irritation of the bronchi (no further details) (BG Chemie 2000).

Inhalation of isoprene at 160 mg/m³ (about 57 ml/m³) by 10 volunteers produced mild mucosal irritation in nose, larynx and pharynx. The odour threshold was cited as being 10 mg/m³ (about 3.6 ml/m³) (no further details) (Gostinskii 1965).

## 4.2 Repeated exposure

The activity of succinate dehydrogenase in immunocompetent blood cells (no further details) was reduced in workers in the rubber industry. The activities of alkaline and acid phosphatases in neutrophils were increased. Apart from isoprene, the workers were exposed to other substances such as styrene, butadiene, isobutylene and chloromethane (Mamedov and Aliev 1985 a, b). These studies are not used for evaluation because of this mixed exposure and a lack of data on the exposure level. In addition, the relevance of changed enzyme activities in immunocompetent cells is debatable.

The upper respiratory tract of 630 workers in isoprene rubber production was investigated between 1965 and 1968. Within the first year of occupation, the workers suffered mainly from catarrh-like nasal inflammation. Thereafter, occupation led increasingly to atrophic processes. Their odour perception was also impaired. Apart from isoprene, the workers were also exposed to formaldehyde and dimethyl

dioxane (Mitin 1969). The results of this study are not meaningful due to mixed exposure at the workplace.

### 4.3 Effects on skin and mucous membranes

Irritation to the skin and eyes (no further details) occurred after application of liquid isoprene (BG Chemie 2000). See Section 4.1 for irritation after inhalation exposure.

### 4.4 Allergenic effects

There are no data available for the allergenic effects of isoprene.

### 4.5 Reproductive and developmental toxicity

There are no data available on reproductive toxicity of isoprene.

### 4.6 Genotoxicity

There are no data available on genotoxicity of isoprene.

### 4.7 Carcinogenicity

There are no data available on carcinogenicity of isoprene.

## 5 Animal experiments and in vitro studies

### 5.1 Acute toxicity

Data on the acute toxicity of isoprene in different species are summarized in Table 3.

**Table 3** Studies on acute toxicity of isoprene after inhalation, oral, dermal, intraperitoneal or subcutaneous administration

| Species, number, sex | Concentration/dose; duration | Endpoint | References |
|---|---|---|---|
| **rat**, Wistar, 20 ♂/♀ per group | ♂: 27 600–100 900 mg/m³ (9893–36 165 ml/m³); 4 h<br>51 500 mg/m³ (18 459 ml/m³); 1 h<br>♀: 29 500–98 100 mg/m³ (10 574–35 161 ml/m³); 4 h | 18 459 ml/m³ (1 h): $LC_{50}$ not reached; 36 165/35 161 ml/m³ (4 h): $LC_{50}$ not reached;<br>transiently poor general condition at the highest concentration | BG Chemie 2000 |
| **rat,** no further details | no further details;4 h | 180 000 mg/m³ (64 516 ml/m³): $LC_{50}$, no further details | Shugaev 1969 |
| **rat,** Wistar, 10 ♂ per group | 0.26; 0.81; 2.18; 4.98; 8.40; 21.44 mg/l (260–21 440 ml/m³); 4 h | at 8400 ml/m³ and above: thymus after 24 h: cell count and mitotic index decreased, absolute and relative weight decreased; reversible after 3 d | Mamedov 1978 |
| **mouse**, NMRI, 10 ♂ per group | 14 100, 31 500 mg/m³ (about 5054, 11 290 ml/m³); 4 h | 11 290 ml/m³: no mortality, no further details | BG Chemie 2000 |
| **mouse**, 10 per group, no further details | 50 000–150 000 mg/m³ (17921–53763 ml/m³); 2 h, recovery period: 21 days,<br>no further details | 53 763 ml/m³: $LC_{50}$ mucosal irritation in the upper respiratory tract, disturbed coordination, lateral recumbency and narcosis, pronounced hyperaemia of the inner organs and of the brain | BG Chemie 2000 |
| **mouse**, ♂, ♀, no further details | no further details;<br>40 min,<br>2 h | at 750 mg/m³ (268 ml/m³) and above (40 min): central nervous effects;<br>139 000 mg/m³ (49 821 ml/m³)/148 000 mg/m³ (53 047 ml/m³) (2 h): ♂/♀: $LC_{50}$, irritating, narcotizing, enlarged lungs and congestion in the lungs | BG Chemie 2000; Gostinskii 1965 |
| **mouse**, no further details | no further details; 2 h | 157 000 mg/m³ (56 272 ml/m³): $LC_{50}$ | Shugaev 1969 |
| **mouse**, no further details | 100 000–120 000 mg/m³ (35 843–50 179 ml/m³); no further details | 35 843–50 179 ml/m³: deep narcosis;<br>50 179 ml/m³: fatal | BG Chemie 2000 |

**Table 3** (Continued)

| Species, number, sex | Concentration/dose; duration | Endpoint | References |
|---|---|---|---|
| **mouse**, Balb/c, 12 ♂ | 0, 465 ml/m$^3$; 60 min exposure, 30 min challenge, 15 min recovery | 465 ml/m$^3$: respiration rate decreased, time of brake and expiratory flow at half tidal volume/tidal volume unchanged | Rohr et al. 2002 |
| **rabbit**, 6, no further details | 190–4100 mg/m$^3$ (68–1470 ml/m$^3$); 40 min | at 68 ml/m$^3$ and above: respiration rate increased by 16–40%; 1470 ml/m$^3$: weakened flexor reflex with rapid onset | BG Chemie 2000; Gostinskii 1965 |
| **cat**, 3, no further details | 400–700 mg/m$^3$ (143–251 ml/m$^3$); 1 h | at 143 ml/m$^3$ and above: latency between stimulus and locomotion reaction increased, rapidity of approach decreased, after 14 d reversible, no further details | BG Chemie 2000 |
| **rat**, Wistar, 15 ♂ per group | 250–2500 mg/kg body weight in vegetable oil; gavage; recovery period: 14 days | 2125 mg/kg body weight (2210–2403 mg/kg body weight): $LD_{50}$ sedation and disturbed breathing within 1 h, which continued up to 7 days, death occurring within 24 h | BG Chemie 2000 |
| **rat**, Wistar, 5 ♂ per group | 1000 µl/kg body weight on the shaved skin of the back; 7 days; recovery period 14 days; no further details | 1000 µl/kg body weight (681 mg/kg body weight): no signs of toxicity, no deaths | BG Chemie 2000 |
| **rat**, Wistar, 15♂ per group | 100–1750 mg/kg body weight in vegetable oil; intraperitoneal; no further details | 1390 mg/kg body weight (1310–1470 mg/kg body weight): $LD_{50}$, sedation and disturbed breathing | BG Chemie 2000 |
| **rabbit**, no further details | 1 ml; subcutaneous; no further details | about 230 mg/kg body weight: leukocytosis, stimulus effect and "anaemia" in the bone marrow, urobilinogen and albumin in urine | BG Chemie 2000 |

### 5.1.1 Inhalation

The $LC_{50}$ of rats after 4-hour inhalation exposure was about 65 000 ml/m$^3$ and that of mice after 2-hour inhalation about 50 000 ml/m$^3$ (BG Chemie 2000). In male rats, reversible effects in the thymus were found 24 hours after 4-hour exposure to 8400 ml/m$^3$ and above (Mamedov 1978). Decreased respiration rates (about 4%) were found in male mice that had been exposed to 465 ml/m$^3$ for 60 minutes. Both the time of brake (pause prior to inhalation) and expiratory flow at half tidal

volume/tidal volume were unchanged (Rohr et al. 2002). An $RD_{50}$ of 57 200 ml/m$^3$ was given for mice (Wilkins et al. 2001; Wolkoff et al. 2000). In mice exposed to 20, 200 or 2000 ml/m$^3$, the respiratory minute volume was significantly reduced at the highest concentration (Bond et al. 1991). In contrast, in another study it had not significantly decreased at 8200 ml/m$^3$ (Dahl et al. 1987). Rabbits exposed up to 1470 ml/m$^3$ showed an increased respiration rate and a flexor reflex which was weaker but had a quicker reaction onset (no further details) (BG Chemie 2000; Gostinskii 1965). Reversible narcotic effects were found in cats after exposure to 143 ml/m$^3$ for one hour (BG Chemie 2000).

### 5.1.2 Ingestion

The oral $LD_{50}$ in male Wistar rats was 2125 mg/kg body weight (BG Chemie 2000).

### 5.1.3 Dermal application

The dermal $LD_{50}$ was given as > 681 mg/kg body weight for male Wistar rats (BG Chemie 2000).

### 5.1.4 Intraperitoneal and subcutaneous administration

The $LD_{50}$ in male Wistar rats was 1390 mg/kg body weight after intraperitoneal injection (BG Chemie 2000).

Leukocytosis, irritant effects, "anaemia" in the bone marrow, and urobilinogen and albumin in the urine were observed in rabbits after subcutaneous injection of 1 ml isoprene (no further details) (BG Chemie 2000).

## 5.2 5.2 Subacute, subchronic and chronic toxicity

### 5.2.1 Inhalation

Data on the toxicity of isoprene after repeated inhalation exposure of rats, mice and rabbits can be found in Table 4.

**Rat**

No mortalities occurred in an NTP (National Toxicology Program) inhalation study with F344/N rats study lasting 14 days. Body weights, haematological and clinico-chemical parameters in the serum, urinalysis and histological investigation showed no abnormal findings (Melnick et al. 1990; NTP 1995).

After 30-day exposure to 35.1 ml/m$^3$, cell proliferation in the thymus was in-

**Table 4** Effects of isoprene after repeated inhalation (whole body exposure)

| Species, strain, no. of animals per sex | Duration, concentration | Findings | References |
|---|---|---|---|
| **rat**, Wistar, 10 ♂/♀ per group | **5 days**, 12 700, 57 300 mg/m$^3$ (about 4547, 20 513 ml/m$^3$), 4 h/day | **20 513 ml/m$^3$: NOAEC**, no further details | BG Chemie 2000 |
| **rat,** Alderley-Park, 2 ♂/♀ per group | **6 days**, 6000 ml/m$^3$, 6 h/day; **15 days**, 1670 ml/m$^3$, 6 h/day | **1670 ml/m$^3$: NOAEC; 6000 ml/m$^3$:** slight congestion of the lungs | Gage 1970 |
| **rat,** F344/N, 10 ♂/♀ per group | **2 weeks**, 0, 438, 875, 1750, 3500, 7000 ml/m$^3$, 6 h/day, 5 days/wk, purity: > 99% | **7000 ml/m$^3$: NOAEC** (mortality, body weight, haematological and clinico-chemical parameters in the serum, histological investigation of liver, lungs including mainstem bronchi, nasal cavity and turbinates, trachea, larynx, tracheobronchial lymph nodes, heart, brain, thymus, spleen, kidneys, testes, epididymides without abnormal findings) | Melnick et al. 1990; NTP 1995 |
| **rat,** Wistar, 10 ♂ per group | **30 days**, 0.098, 1.016 mg/l (about 35.1; 364.2 ml/m$^3$), 4 h/day (no further details) | **35.1 ml/m$^3$:** cell proliferation in the thymus increased; **364.2 ml/m$^3$:** cell count and mitotic index in the thymus decreased | Mamedov 1978 |
| **rat,** Wistar, 10 ♂ per group | **4 months**, 0.0108; 0.116 mg/l (about 3.9; 41.6 ml/m$^3$), 4 h/d, 5 days/wk, 1 month recovery period | **41.6 ml/m$^3$: after** 4 months: thymus weight increased, cell count and mitotic index in the thymus increased, changes in lymphocyte count (no further details); after 5 months: cell proliferation in the thymus increased | Mamedov 1978 |

**Table 4** (Continued)

| Species, strain, no. of animals per sex | Duration, concentration | Findings | References |
|---|---|---|---|
| **rat,** F344/N, 10 ♂/♀ per group | **13 weeks**, 0, 70, 220, 700, 2200, 7000 ml/m$^3$, 6 h/day, 5 days/wk, purity: > 99% | **7000 ml/m$^3$: NOAEC,** changes in organ weight and changed parameters in blood and urine not substance-related, no mortality, unchanged body weight and body weight gain, histological studies without abnormal findings | Melnick et al. 1994; NTP 1995 |
| **rat**, F344/N, 40 ♂ per group | **6 months**, 0, 70, 220, 700, 2200, 7000 ml/m$^3$, 6 h/day, 5 days/wk, per group 10 animals investigated; recovery period 6 months (per group 30 ♂), purity: > 99% | **after 6 months**: **7000 ml/m$^3$**: Leydig cell hyperplasia (10/10, control: 1/10); **after 12 months (6 months exposure and 6 months recovery period)**: **at 70 ml/m$^3$ and above**: Leydig cell hyperplasia in all exposed groups: 30/30, control: 25/30 | Melnick et al. 1992, 1994, 1996; NTP 1995 |
| **rat**, F344/N, 50 ♂/♀ per group | **105 weeks**, 0, 220, 700, 7000 ml/m$^3$, 6 h/day, 5 days/wk | **at 700 ml/m$^3$ and above**: ♂: fibrotic changes in the spleen, renal tubular hyperplasia; **7000 ml/m$^3$**: ♂: hyperplasia in the parathyroid gland, ♀: purulent inflammation in the nose, bile duct hyperplasia (compare Section 5.7.2) | NTP 1999 |
| **mouse**, B6C3F$_1$, per group 10 ♂/♀ | **2 weeks**, 0, 438, 875, 1750, 3500, 7000 ml/m$^3$, 6 h/day, 5 days/wk, purity: > 99% | **at 438 ml/m$^3$ and above**: ♂, ♀: haematocrit decreased, haemoglobin decreased, erythrocyte count decreased, relative liver weight increased, absolute thymus weight decreased, epithelial hyperplasia in the forestomach; ♂: body weight decreased, cytoplasmic vacuolization of hepatocytes; absolute and relative spleen weight decreased, absolute testis weight decreased; ♀: absolute liver weight increased, relative thymus weight decreased; **at 875 ml/m$^3$ and above**: ♂: absolute liver weight increased, relative thymus- and testis weight decreased; **at 1750 ml/m$^3$ and above**: ♂: degeneration of the olfactory epithelium; | Melnick et al. 1990; NTP 1995 |

**Table 4** (Continued)

| Species, strain, no. of animals per sex | Duration, concentration | Findings | References |
|---|---|---|---|
| | | **at 3500 ml/m³ and above**: ♀: absolute and relative spleen weight decreased;<br>**7000 ml/m³**: ♂: body weight gain decreased, atrophy of the thymus and of the testes | |
| **mouse**, no further details | **20 days**, 60 000 mg/m³ (about 21 503 ml/m³), 2 h/day no further details | irritation of bronchi, pulmonary emphysema, hyperplasia of the bone marrow, signs of increased erythrocyte turnover in the spleen (no further details) | BG Chemie 2000 |
| **mouse**, $B6C3F_1$, 10 ♂/♀ per group | **13 weeks**, 0, 70, 220, 700, 2200, 7000 ml/m³, 6 h/day, 5 days/wk, purity: > 99% | **70 ml/m³**: ♀: body weight gain not concentration-dependently decreased;<br>**220ml/m³**: ♂: relative spleen weight decreased, ♀: absolute kidney weight increased;<br>**at 700 ml/m³ and above**: ♂, ♀: haematocrit decreased, haemoglobin decreased, erythrocytes decreased, mean cell volume increased, means cell haemoglobin increased, macrocytic anaemia, absolute spleen weight decreased, epithelial hyperplasia in the forestomach<br>**at 2200 ml/m³ and above**: ♂: Howell-Jolly bodies increased, cytoplasmic vacuolization in the liver, absolute and relative testis weight decreased;<br>**7000 ml/m³**: ♂: relative liver weight increased, degeneration of the olfactory epithelium, sperm concentration, spermatid heads decreased, testicular atrophy,<br>♀ : relative spleen weight decreased, oestrous cycle length increased; ♂, ♀: glutathione concentration in liver and lung decreased, absolute liver weight increased;<br>no mortality, body weight gain without abnormal findings | Melnick et al. 1994; NTP 1995 |
| **mouse**, $B6C3F_1$, 40 ♂ per group | **6 months,** 0, 70, 220, 700, 2200, 7000 ml/m³, 6 h/day, 5 day/wk, per group | **after 6 months**:<br>**70 ml/m³**: NOAEC;<br>**at 220 ml/m³ and above**: grip strength of fore- and hindlimbs decreased; | Melnick et al. 1992, 1994, 1996; NTP 1995 |

**Table 4** (Continued)

| Species, strain, no. of animals per sex | Duration, concentration | Findings | References |
|---|---|---|---|
| | 10 animals investigated; recovery period 6 months (per group 30 ♂), purity: > 99% | **at 700 ml/m³ and above**: epithelial hyperplasia of the forestomach, macrocytic anaemia;<br>**7000 ml/m³**: mortality increased, impaired hindlimb function, degeneration of the olfactory epithelium in nasal turbinates, degeneration in white matter of spinal cord, atrophy of skeletal muscles and testes<br>**6 months exposure and 6 months recovery period**:<br>**at 70 ml/m³ and above**: degeneration the white matter of spinal cord;<br>**at 220 ml/m and above ³**: degeneration of the olfactory epithelium in nasal turbinates;<br>**at 700 ml/m³ and above**: epithelial hyperplasia in the forestomach and the alveoli, hepatocellular foci increased;<br>**7000 ml/m³**: mortality increased, tumour incidence increased; compare Section 5.7.2 | |
| **mouse**, $B6C3F_1$, 50 ♂ per group | **80 weeks**, 0, 10, 70, 280, 700, 2200 ml/m³, 8 h/day, 5 days/wk, (2200 ml/m³, 4 and 8 h/day), purity: ≥ 99% | **at 10 ml/m³ and above**: proliferation of haematopoietic cells in the spleen, myeloid hyperplasia in the bone marrow;<br>**at 280 ml/m³ and above**: survival < 50% (no further details),<br>absolute and relative testis weight decreased, mild metaplasia of the olfactory epithelium to respiratory epithelium;<br>tumours compare Section 5.7.2 | Placke et al. 1996 |
| **mouse**, $B6C3F_1$, 50 ♀ per group | **80 weeks**, 0, 10, 70 ml/m³, 8 h/day, 5 days/wk, purity: ≥ 99% | **at 10 ml/m³ and above**: proliferation of haematopoietic cells in the spleen, myeloid hyperplasia in the bone marrow;<br>**70 ml/m³**: slight metaplasia of the olfactory epithelium into respiratory epithelium;<br>for tumours see Section 5.7.2 | Placke et al. 1996 |
| **rabbit**, (no further details) | **4 months**, 0, 400 mg/m³ (143 ml/m³), 4 h/day (no further details), room temperature or 30–32°C | **143 ml/m³**: at room temperature: activity of immune capacities decreased (no further details);<br>at 32°C: inhibition of immunological reaction ability (no further details) | Samedov et al. 1978 |

**Table 4** (Continued)

| Species, strain, no. of animals per sex | Duration, concentration | Findings | References |
|---|---|---|---|
| **rabbit**, (no further details) | **3–4 months**, 400 mg/m³ (143 ml/m³), no further details | **143 ml/m³**: phagocyte count decreased (no further details) | Faustov 1972 |

creased in male Wistar rats and cell count and mitotic index in the thymus were decreased at 364.2 ml/m³ (Mamedov 1978). Thymus weight, cell count and mitotic index in the thymus were increased and the lymphocyte count changed (no other details) after 4-month treatment at 41.6 ml/m³. Except cell proliferation, all parameters had normalized after a treatment free period lasting one month (Mamedov 1978).

In the NTP study, male and female F344/N rats were whole-body exposed to isoprene for 13 weeks. A reduced neutrophil count in the females at 70 ml/m³ and above was also found in the males of the 7000 ml/m³ group. According to the authors, this effect could be compatible with a shift of neutrophils from the circulating pool into the marginal pool, as neither a decrease in leukocyte count nor a change in the bone marrow cellularity counts could be found. The authors consider all changes in organ weight and the changed parameters in blood and urine not to be substance-related. No mortality occurred. No abnormal findings were obtained in the context of body weight, body weight gain, histological investigation of the organs, determination of sperm motility and vaginal cytology (compare Section 5.5.1) (Melnick et al. 1994; NTP 1995).

In a further NTP study, male F344/N rats were exposed for six months. The incidence of Leydig cell hyperplasia in the testes was increased by the end of exposure at 7000 ml/m³ and at the end of the recovery period in the animals of all concentration groups.

In the rats of the 7000 ml/m³ recovery period group, Leydig cell adenomas occurred (see Section 5.7.2). Body weight, body weight gain and haematological parameters were unchanged by treatment (Melnick et al. 1992, 1994, 1996; NTP 1995). This means that the lowest used concentration of 70 ml/m³ is also the LOAEC.

In an NTP carcinogenicity study, male and female F344/N rats inhaled 0, 220, 700 or 7000 ml/m³ for 105 weeks (see Section 5.7.2). Fibrotic changes in the spleen and renal tubular hyperplasia were observed in the males at 700 ml/m³ and above. Hyperplasia in the parathyroid gland occurred with increased frequency in the males, and purulent inflammation in the nose and hyperplasia in the bile duct in

the females at 7000 ml/m$^3$. Body weight, body weight gain and survival rate were unchanged in the treated animals (NTP 1999).

Two studies with rats exposed for five and six months cannot be evaluated due to inadequate data (sex, strain, control animals, time of the occurrence of effects) and inadequate documentation (BG Chemie 2000).

### Mouse

A 20 times 2-hour exposure to isoprene at 60 000 mg/m$^3$ (about 21 505 ml/m$^3$) produced no narcotic effects in mice (no further details). Macroscopic assessment revealed bronchial irritation and pulmonary emphysema in some animals. Histopathology showed hyperplasia of the bone marrow and signs of red blood cell degeneration in the spleen (pigmentation, macrophages; no further details) (BG Chemie 2000).

In a whole-body exposure study with B6C3F$_1$ mice by the NTP lasting 14 days, a decrease in haematocrit values, haemoglobin concentrations and erythrocyte counts was observed in males and females at 438 ml/m$^3$ and above. This indicates mild intravascular or extravascular haemolysis. Cytoplasmic vacuolization in the liver was observed in the males at an isoprene concentration of 438 ml/m$^3$ and above. Liver histopathology was without findings in the females. Epithelial hyperplasia in the forestomach developed in both sexes. Liver weights were increased, spleen, thymus and testis weights decreased. Concentration-dependent degeneration of the olfactory epithelium was found in the males at 1750 ml/m$^3$ and above. Atrophy of the testes and the thymus was diagnosed at 7000 ml/m$^3$ (Melnick et al. 1990; NTP 1995). No NOAEC was obtained.

In a further NTP study, B6C3F$_1$ mice were whole-body exposed for 13 weeks. Macrocytic anaemia and epithelial hyperplasia of the forestomach was found in males and females at 700 ml/m$^3$ and above. The latter was occasionally associated with intraepithelial microabscesses and infiltrates of different inflammatory cells from the submucosa. Decreased absolute and relative testis weights were found at 2200 ml/m$^3$ and above. Degenerations of the olfactory epithelium, testicular atrophy with decreased sperm counts and increased liver weights were found in the male mice of the 7000 ml/m$^3$ group. In the females, this dose produced spleen and liver weight changes and increased oestrous cycle length. The glutathione concentration in lungs and liver was reduced in both males and females (NTP 1995; Melnick et al. 1994). As the body weight gain was still reduced at 70 ml/m$^3$ in the female mice, no NOAEC can be given.

In a further NTP study involving male B6C3F$_1$ mice with 6-month exposure and a 6-month recovery period, a reversible reduction in the grip strength of fore- and hindlimbs at 220 ml/m$^3$ and above was found after six months. Epithelial hyperplasia of the forestomach was found at 700 ml/m$^3$ and above, which was still present after the 6-month exposure-free period. The macrocytic anaemia observed at 700 ml/m$^3$ and the atrophy of skeletal muscles and testes at 7000 ml/m$^3$ were reversible after six months. Increased mortality occurred at 7000 ml/m$^3$ also during the recovery period.

Degeneration of the spinal cord occurred at 70 ml/m$^3$ and above and degeneration of the olfactory epithelium in the turbinates at 220 ml/m$^3$ and above after 12 months (6-month exposure and 6-month recovery period). Hyperplasia of the alveolar and forestomach epithelia was found at 700 ml/m$^3$ and above (Melnick et al. 1992, 1994, 1996; NTP 1995). No NOAEC can be given for this study, as degeneration of the spinal cord occurred during the recovery period at 70 ml/m$^3$. Tumours are described in Section 5.7.2.

In a study with male and female B6C3F$_1$ mice, proliferation of the haematopoietic cells in the spleen and myeloid hyperplasia in the bone marrow were found after 80-week exposure to 10 ml/m$^3$ and above. At 70 ml/m$^3$ (males) or 280 ml/m$^3$ (females) and above, a mild metaplasia of the olfactory epithelium to respiratory epithelium occurred. Tumours are described in Section 5.7.2 (Placke et al. 1996). In total, no NOAEC can be given for the study.

#### Rabbit

Although three inhalation studies with rabbits are available (BG Chemie 2000; Faustov 1972; Samedov et al. 1978), they are unsuitable for evaluation due to insufficient documentation of study design and results.

### 5.2.2 Ingestion

Isoprene was administered to groups of 30 male Wistar rats in doses of 200 mg/kg body weight and day on the first day, and 45 mg/kg body weight and day over the subsequent four days (no other details). The animals were observed for another seven days. No deaths occurred. No cumulative effect could be found (no further details; BG Chemie 2000).

### 5.2.3 Dermal application

There are no data available for dermal application of isoprene.

## 5.3 Effects on skin and mucous membranes

Isoprene was applied with a brush to one ear each of two White New Zealand rabbits twice daily on five consecutive days. This produced short-term reddening of the skin. A low potential for skin damage was therefore assessed by the authors (BG Chemie 2000).

Application of 0.5 ml isoprene to the shaved skin of a rabbit resulted in pronounced hyperaemia, oedema formation and subsequent desquamation (no further details; BG Chemie 2000).

The tail skin of mice was moistened with isoprene. Within two hours, a pronounced hyperaemia of the skin was observed and, on the following days, necrosis of the tail tips (no further details; BG Chemie 2000).

It has been reported that isoprene is able to produce irritation of the eyes in rats (OECD 2005).

## 5.4 Allergenic effect

There are no data available for the allergenic effect of isoprene.

## 5.5 Reproductive toxicity

### 5.5.1 Fertility

Fertility studies or investigations on the reproductive organs are shown in Table 5.

In the 13 weeks inhalation studies with F344/N rats and B6C3F$_1$ mice at isoprene concentrations of 0, 70, 700 and 7000 ml/m$^3$, the weight of epididymides and testes, the number of spermatids and sperm heads, and the concentration and motility of the sperms in the epididymides were determined. In addition, a characterization of the oestrous cycle was carried out. The NOAEC for changes in the reproductive organs of rats was 7000 ml/m$^3$. In contrast, the absolute weight of the epididymides and of the tail section of the epididymides (*cauda epididymidis*), sperm motility, sperm concentration, number of spermatids and sperm heads per testes were decreased at 700 ml/m$^3$ and above in the male mice. Decreased absolute testis weight and testicular atrophy occurred at 7000 ml/m$^3$. At this concentration, oestrous cycle length was increased in the females. Therefore, the NOAEC for changes in the reproductive organs is 70 ml/m$^3$ for mice (Melnick et al. 1994; NTP 1995; compare Section 5.2.1).

Histological investigation of the testes after 6-month inhalation exposure to isoprene at 7000 ml/m$^3$ revealed hyperplasia of the interstitial cells in male rats. At this concentration, interstitial cell adenomas were observed after the 6-month recovery period (Melnick et al. 1992, 1994, 1996; NTP 1995). Decreased absolute and relative testis weight and testicular atrophy occurred in mice after six months of exposure to 7000 ml/m$^3$ but not at the end of the 6-month recovery period (Melnick et al. 1992, 1994, 1996; NTP 1995; see Section 5.2.1).

One inhalation study in rats cannot be evaluated due to insufficient data (strain, number of animals, control animals) and only one concentration used (Repina 1988).

In mice, intraperitoneal administration of 7.34 mmol/kg body weight and day (about 500 mg/kg body weight and day) for 30 days produced a reduced number of

**Table 5** Fertility studies with isoprene

| Species | Exposure | Concentration: findings | References |
|---|---|---|---|
| **rat**, no data on strain, ♂, no data on number | **4 hours**, 4000 mg/m$^3$ (about 1434 ml/m$^3$), whole-body exposure, controls: no data | **1434 ml/m$^3$**: no effects on spermatozoa 24 h after end of exposure (number of spermatozoa, percentage of living, motile and pathological spermatozoa; no further details) | Repina 1988 |
| **rat**, F344/N, 10 ♂/♀ per group | **13 weeks**, 0, 70, 700, 7000 ml/m$^3$, whole-body exposure, 6 h/day, 5 days/wk, purity: > 99% | **7000 ml/m$^3$**: **NOAEC** (sperm motility, vaginal cytology; compare Section 5.2.1) | Melnick et al. 1994; NTP 1995 |
| **rat**, F344/N, 40 ♂ per group | **6 months**, 0, 70, 220, 700, 2200, 7000 ml/m$^3$, 6 h/day, 5 days/wk, per group 10 animals investigated; recovery period 6 months (per group 30 ♂), purity: > 99% | **after 6 months**: **7000 ml/m$^3$**: hyperplasia of interstitial cells of the testes (10/10, control: 1/10); **6-month exposure and 6-month recovery period**: **at 700 ml/m$^3$ and above**: hyperplasia of interstitial cells of the testes (30/30, control: 25/30); **7000 ml/m$^3$**: interstitial cell adenomas of the testes; see Section 5.2.1, Section 5.7.2 | Melnick et al. 1992, 1994, 1996; NTP 1995 |
| **mouse**, B6C3F$_1$, 10 ♂/♀ per group | **13 weeks**, 0, 70, 700, 7000 ml/m$^3$, whole-body exposure, 6 h/day, 5 days/wk, purity: > 99% | **70 ml/m$^3$**: **NOAEC**; **at 700 ml/m$^3$ and above**: ♂: absolute weight of epididymides and cauda epididymides decreased, sperm motility, sperm concentration, number of spermatids, sperm head count per testes decreased; **7000 ml/m$^3$**: ♂: absolute testis decreased, testicular atrophy (2/10), ♀: length of oestrous cycle increased; see Section 5.2.1 | Melnick et al. 1994; NTP 1995 |
| **mouse**, B6C3F$_1$, 40 ♂ per group | **6 months**, 0, 70, 220, 700, 2200, 7000 ml/m$^3$, 6 h/day, 5 days/wk, per group 10 animals investigated; recovery period 6 months (per group 30 ♂), purity: > 99% | **after 6 months**: **7000 ml/m$^3$**: absolute and relative testicular weight decreased, testicular atrophy; **6 months exposure and 6 months recovery period**: **7000 ml/m$^3$**: testicular weight and histopathology of the testes without abnormal findings; see Section 5.2.1 | Melnick et al. 1992, 1994, 1996; NTP 1995 |

**Table 4** (Continued)

| Species | Exposure | Concentration: findings | References |
|---|---|---|---|
| **mouse**, $B6C3F_1$, 10 ♀ per group | **30 days**, 0, 7.34 mmol/kg body weight and d (about 500 mg/kg body weight and day), intraperitoneal, once/day, controls: sesame oil | **500 mg/kg body weight and day**: number of small and growing follicles decreased | Doerr et al. 1995 |

small (primordial) and growing (primary up to preantral) follicles in the ovaries (Doerr et al. 1995).

### 5.5.2 Developmental toxicity

Studies of the developmental toxicity of isoprene are given in Table 6.

**Table 6** Developmental toxicity studies with isoprene

| Species strain, no. of animals | Exposure | Concentration or dose: findings | References |
|---|---|---|---|
| **rat**, Sprague Dawley, 24–26 ♀ per group | **gestation days 6–19**, 0, 280, 1400, 7000 ml/$m^3$ whole-body exposure, 6 h, 7 days/wk, investigated on gestation day 20, purity: >99% | **7000 ml/$m^3$**:<br>foetuses: **NOAEC**;<br>dams: **NOAEC** for maternal toxicity (with regard to mortality and body weight gain) | Mast et al. 1990; NTP 1995 |
| **mouse**, CD-1 Swiss, 28–30 ♀ per group | **gestation days 6–17**, 0, 280, 1400, 7000 ml/$m^3$ whole-body exposure, 6 h, 7 days/wk, investigated on gestation day 18, purity: >99% | **at 280 ml/$m^3$ and above**:<br>foetuses: ♀ foetus weight decreased, **NOAEC**;<br>**1400 ml/$m^3$**:<br>dams: **NOAEC** for maternal toxicity;<br>**at 1400 ml/$m^3$ and above**:<br>foetuses: ♂ foetuses weight decreased;<br>**7000 ml/$m^3$**:<br>foetuses: foetuses per litter with variations/reduced ossification (mostly supernumerary ribs) increased;<br>dams: body weight gain decreased, uterus weight (gravid uterus) decreased, absolute and relative kidney weight increased | Mast et al. 1990; NTP 1995 |

**Table 6** (Continued)

| Species strain, no. of animals | Exposure | Concentration or dose: findings | References |
|---|---|---|---|
| **rat**, Wistar, 10 ♀ per group | **gestation days 9–12**, 0, 22, 379, 1895 mg/kg body weight and day, oral (no further details), time of study: no data, purity: no data | **at 22 mg/kg body weight and above**: foetuses: number of resorptions increased (not dose-dependently), reduced ossification of the sternum and of the occipital bone increased (also not dose-dependently); **up to 1895 mg/kg body weight**: foetuses: no skeletal and no external malformations: dams: body weight gain not restricted | Komatsu 1971 |

No clear signs of maternal toxicity were found in pregnant Sprague Dawley rats after inhalation of isoprene at 0, 280, 1400 or 7000 ml/m$^3$ from days 6 to19 of gestation. Only the relative kidney weights were increased. In addition, no noticeable findings were observed in the pregnancy, survival and body weight parameters. No external, skeletal and visceral malformations in the foetuses were found at any of the concentrations. Also, the total incidence of foetuses per litter with variations/reduced ossification was unaffected. However, it is mentioned that the mean percentage of foetuses per litter with reduced vertebral ossification increased with increasing concentration, but none of these percentages are detailed. According to the authors, no maternal toxicity and no developmental toxicity occurred in this strain up to 7000 ml/m$^3$ (Mast et al. 1990; NTP 1995). A NOAEC of 7000 ml/m$^3$ is thus assumed for maternal toxicity and developmental toxicity from this study.

After inhalation of isoprene at 0, 280, 1400 or 7000 ml/m$^3$ from days 6 to 17 of gestation, body weight gain was decreased and absolute and relative kidney weights were increased in pregnant CD-1 Swiss mice at 7000 ml/m$^3$. There was a concentration-dependent, significant decrease in the body weight of the female foetuses of all concentration groups (see Table 7), and of male foetuses at 1400 ml/m$^3$ and above. The percentage of foetuses per litter with variations/reduced ossification (mostly supernumerary ribs) was increased at 7000 ml/m$^3$ (see Table 7).

A concentration of 1400 ml/m$^3$ is obtained from this study as the NOAEC for maternal toxicity. However, haematotoxic and hepatotoxic effects of isoprene are to be expected at lower concentrations, but they were not recorded (Mast et al. 1990; NTP 1995). In a study with B6C3F$_1$ mice lasting 13 weeks, a NOAEC of 220 ml/m$^3$ was stated (Mast et al. 1990; NTP 1995). In regard to the reduced foetal weights, however, it must be taken into account that the dams of the 280 ml/m$^3$ group gave birth to more living foetuses on average than the control animals. This can result in a reduction in the weight of the foetuses and simulate or conceal an exposure-produced effect. The NOAEC is thus considered to be 280 ml/m$^3$.

**Table 7** Findings in foetuses of the developmental toxicity study with isoprene in mice (Mast et al. 1990; NTP 1995;)

| Endpoint | | Isoprene concentration (ml/m³) | | | |
|---|---|---|---|---|---|
| | | 0 | 280 | 1400 | 7000 |
| Number of investigated dams/litters | | 28 | 29 | 28 | 27 |
| Living foetuses/litter (M±SD) | | 11.5 ± 3.0 | 12.0 ± 1.9 | 11.9 ± 2.2 | 10.9 ± 1.8 |
| Dead foetuses/litter (M±SD) | | 0.0 ± 0.0 | 0.1 ± 0.3 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Foetal weight (g) | ♂ | 1.37 ± 0.11 | 1.30 ± 0.10 | 1.23 ± 0.10* | 1.16 ± 0.12* |
| | ♀ | 1.32 ± 0.10 | 1.25 ± 0.10* | 1.20 ± 0.10* | 1.12 ± 0.13* |
| Variations or reduced ossification | | | | | |
| Foetuses with variations or reduced ossification (n) | | 48 (13.4%) | 40 (11.5%) | 46 (13.1%) | 55 (17.5%) |
| Litters with variations or reduced ossification (n) | | 16 (61.5%) | 16 (64.0%) | 16 (64.0%) | 17 (70.8%) |
| Foetuses per litter with variations/ reduced ossification (mostly supernumerary ribs)[a)] (M±SD) | | 24.0 ± 25.6 | 25.3 ± 27.0 | 36.4 ± 26.4 | 41.3 ± 21.8* |

* $p < 0.05$ (Tukey's t test);
[a)] no further data given;
M ± SD = mean ± standard deviation

In a study published in Japanese, ten pregnant rats per group were given isoprene orally in doses of 0, 22, 379 or 1895 mg/kg body weight and day from days 9 to 12 of gestation. The body weight gain of the dams was not affected. The resorption frequencies (4.8, 3.1 and 6.3%) were higher than in controls, which appeared to be exceptionally low at 0%.

The average body weight of surviving foetuses was reduced in the low, but not in the high dose groups. Therefore, no substance-specific effect is to be assumed. In the foetuses of the isoprene-exposed dams, the occurrence of reduced sternal ossification was markedly more frequent than in the controls (16.7%), but not dose-dependent (70.2%, 41%, 65%). The number of foetuses with reduced ossification of the occipital bone was also higher than in the controls (0%) although, here too, no dose-dependency was found (7%, 0%, 3.3%). No external, skeletal or visceral malformations were observed (Komatsu 1971). Evaluation of the findings is difficult because the control values seem to be exceptionally low and there is no dose-dependency in the case of possible effects. In addition, oral administration of a readily volatile substance is to be questioned. This study is thus not used to evaluate the developmental toxicity of isoprene.

## 5.6 Genotoxicity

### 5.6.1 In vitro

Studies on the in vitro genotoxicity of isoprene are given in Table 8 and those with its metabolites in Table 9.

**Table 8** Studies on the genotoxicity of isoprene in vitro

| Test system | | Concentration; purity; solvent | Results | | Cyto toxicity | References |
|---|---|---|---|---|---|---|
| | | | – m.a. | + m.a. | | |
| BMT | *S. typhimurium* TA102, TA104 | no further details; gas phase | – | n.p. | no data | Kushi et al. 1985 |
| BMT | *S. typhimurium* TA98, TA100, TA1530, TA1535, TA1538 | 25% v/v (TA1530 with 75% isoprene v/v); 99%; gas phase | – | – | no data | de Meester et al. 1981 |
| BMT (preincubation) | *S. typhimurium* TA98, TA100, TA1535, TA1537 | 100–10 000 µg/plate; > 99%; DMSO | – | – | 10 000 µg/plate | Mortelmans et al. 1986 |
| BMT (plate incorporation) | *S. typhimurium* TA98, TA100, TA1535, TA1537 | 100–10 000 µg/plate; > 99%; DMSO | – | – | 10 000 µg/plate | NTP 1983, 1995, 1999 |
| SCE | CHO cells | 50–1600 µg/ml (–m.a.); 160–5000 µg/ml (+ m.a.); > 99%; DMSO | – | – | no data | NTP 1995, 1999 |
| CA | CHO cells | 1600–5000 µg/ml; > 99%; DMSO | – | – | no data | NTP 1995, 1999 |

BMT: bacterial mutagenicity test, CA: chromosome aberration, DMSO: dimethyl sulfoxide, m.a.: metabolic activation, n.p.: not performed, SCE: sister chromatid exchange

**Isoprene**

In a number of bacterial mutagenicity tests with different *S. typhimurium* strains isoprene (as gas or dissolved in dimethyl sulfoxide) produced no mutations in the presence and absence of a metabolic activation system (Kushi et al. 1985; de Meester et al. 1981; Mortelmans et al. 1986; NTP 1983, 1995, 1999).

**Table 9** Studies on the genotoxicity of isoprene metabolites in vitro

| Test system | | Concentration; purity; solvent | Results | | Cyto toxicity | References |
|---|---|---|---|---|---|---|
| | | | – m.a. | + m.a. | | |
| BMT (plate incorporation) | *S. typhimurium* TA98, TA100 | 7.5–30 mM 1,2-epoxy-2-methyl-3-butene; 95%; DMSO | – | n. p. | 30 mM (TA100), – (TA98) | Gervasi et al. 1985 |
| BMT (plate incorporation) | *S. typhimurium* TA98, TA100 | 5–30 mM 1.2-epoxy-3-methyl-3-butene; no data on purity; DMSO | – | n. p. | 30 mM | Gervasi et al. 1985 |
| BMT (plate incorporation) | *S. typhimurium* TA98, TA100 | 2–30 mM 1,2:3,4-diepoxy-2-methyl butane; 99%; DMSO | + at 7.5 mM/plate and above in TA100 | n. p. | 30 mM | Gervasi et al. 1985 |

BMT: bacterial mutagenicity test, DMSO: dimethyl sulfoxide,
m. a.: metabolic activation, n.p.: not performed

Negative results were obtained with isoprene in the SCE test with CHO cells up to concentrations of 1600 µg/ml (without addition of a metabolic activation system) or up to 5000 µg/ml (with addition of a metabolic activation system) (NTP 1995, 1999). There were also no increased incidences of chromosome aberrations up to 5000 µg/ml in CHO cells (NTP 1995, 1999).

#### Metabolites of isoprene

The monoepoxides 1,2-epoxy-2-methyl-3-butene and 1,2-epoxy-3-methyl-3-butene produced no mutations in *Salmonella typhimurium* strains TA98 and TA100 without addition of a metabolic activation system up to 30 mM/plate. The diepoxide 1,2:3,4-diepoxy-2-methylbutane showed mutagenic effects in *Salmonella typhimurium* strain TA100 without addition of a metabolic activation system (Table 9; Gervasi et al. 1985).

### 5.6.2 In vivo

Table 10 shows the studies on the in vivo genotoxicity of isoprene. After 12-day inhalation exposure to isoprene increased SCE frequencies occurred in the bone marrow of male mice at 220 ml/m$^3$ and above; no further increase in SCE frequencies was observed at about 700 ml/m$^3$ and above (Shelby 1990; Tice 1988; Tice et al. 1988).

**Table 10** Studies on the genotoxicity of isoprene in vivo

| Test system | | Duration, mode of administration, concentration | Results | References |
|---|---|---|---|---|
| SCE | mouse, $B6C3F_1$, 4 ♂ per group | **12 days**, inhalation, 0, 438, 1750, 7000 ml/m$^3$; 6 h/day (3 days exposure, 2 days exposure-free, 5 days exposure, 2 days exposure-free, 4 days exposure) | 17–20 h after end of exposure: bone marrow cells: + at and above 438 ml/m$^3$ bone marrow cells: average generation time at 7000 ml/m$^3$ increased; mitotic index unchanged | Tice 1988; Tice et al. 1988 |
| SCE | mouse, $B6C3F_1$, 4 ♂ per group | **12 days**, inhalation, 0, 70, 220, 700 ml/m$^3$; 6 h/day (3 days exposure, 2 days exposure-free, 5 days exposure, 2 days exposure-free, 4 days exposure) | 17–20 h after end of exposure: bone marrow cells: + at and above 220 ml/m$^3$, concentration-dependent | Shelby 1990 |
| mutations | mouse, $B6C3F_1$, 40 ♂ per group | **26 weeks**, inhalation, 0, 2200, 7000 ml/m$^3$; 6 h/day, 5 days/wk, recovery period: 26 w | in tumours of **Harderian gland**: 60% K-ras-mutations and 40% H-ras-mutations (spontaneous tumours in controls: 8% K-ras and 48% H-ras); A→T transversions in K-ras codon 61: 15/30 and C→A transversions in H-ras codon 61: 8/30 (spontaneous tumours in controls: 2/27 K-ras and 4/25 H-ras); increased proliferating cell nuclear antigen index in the tumours compared with spontaneous tumours; in **lung** tumours: A→T transversions at K-ras codon 61: 10/11 (spontaneous tumours in controls: 0/82 K-ras); in **forestomach** tumours: G→C transversions at K-ras codon 13: 5/10 and A→T transversions at H-ras codon 61: 2/10 (spontaneous tumours in controls: 1/11 K-ras and 0/11 H-ras) | Hong et al. 1997; Sills et al. 1999 a, b, 2001 |

**Table 10** (Continued)

| Test system | | Duration, mode of administration, concentration | Results | References |
|---|---|---|---|---|
| MN | rat, F344/N, 10 ♂, ♀ per group | **4 weeks**, inhalation, 0, 220, 700, 7000 ml/m$^3$; 6 h/day, 5 days/wk; purity: > 99% no positive controls | lung fibroblasts: – | NTP 1999 |
| MN | mouse, B6C3F$_1$, 15 ♂ per group | **12 days**, inhalation, 0, 438, 1750, 7000 ml/m$^3$; 6 h/day (3 days exposure, 2 days exposure-free, 5 days exposure, 2 days exposure-free, 4 days exposure) | PCEs, NCEs in peripheral blood: + at and above 438 ml/m$^3$ 23 h after end of exposure | Shelby and Witt 1995; Tice 1988; Tice et al. 1988 |
| MN | mouse, B6C3F$_1$, 15 ♂ per group | **12 days**, inhalation, 0, 70, 220, 700 ml/m$^3$; 6 h/day (3 days exposure, 2 days exposure-free, 5 days exposure, 2 days exposure-free, 4 days exposure) | PCEs, NCEs in peripheral blood: + at 700 ml/m$^3$ 23 h after end of exposure | Shelby 1990 |
| MN | mouse, B6C3F$_1$, 10 ♂, ♀ per group | **13 weeks**, inhalation, 0, 70, 220, 700, 2200, 7000 ml/m$^3$; 6 h/day, 5 days/wk; purity: > 99% no positive controls | ♂: PCEs, NCEs in peripheral blood: + at 700 ml/m$^3$ and above; ♀: PCEs, NCEs in peripheral blood: + at 220 ml/m$^3$ and above | NTP 1999 |
| MN | mouse, B6C3F$_1$, 10 ♂ per group | **40 weeks**, inhalation, 0, 70, 140, 2200 ml/m$^3$; 8 h/day, 5 days/wk | 24 h after end of exposure: PCEs in peripheral blood: + at 2200 ml/m$^3$ | Placke et al. 1996 |

**Table 10** (Continued)

| Test system | | Duration, mode of administration, concentration | Results | References |
|---|---|---|---|---|
| MN | mouse, $B6C3F_1$ 10 ♂ per group | **80 weeks**, inhalation, 0, 10, 70, 280, 700, 2200 ml/m$^3$; 8 h/day (at 2200 ml/m$^3$ additional: 4 h/day), 5 days/wk | 24 h after end of exposure: PCEs in peripheral blood: + at and above 700 ml/m$^3$ (also at 2200 ml/m$^3$ after 4 h) | Placke et al. 1996 |
| CA | mouse, $B6C3F_1$, 8 ♂ per group | **12 days**, inhalation, 0, 438, 1750, 7000 ml/m$^3$; 6 h/day (3 days exposure, 2 days exposure-free, 5 days exposure, 2 days exposure-free, 4 days exposure) | 17–20 h after end of exposure: bone marrow cells: – | Shelby and Witt 1995; Tice 1988; Tice et al. 1988 |
| CA | mouse, $B6C3F_1$, 8 ♂ per group | **12 days**, inhalation, 0, 70, 220, 700 ml/m$^3$; 6 h/day (3 days exposure, 2 days exposure-free, 5 days exposure, 2 days exposure-free, 4 days exposure) | 17–20 h after end of exposure: bone marrow cells: – | Shelby 1990 |

CA: chromosome aberration, MN: micronucleus test,
NCE: normochromatic erythrocytes, PCE: polychromatic erythrocytes,
SCE: sister chromatid exchange

Increased frequencies of K-ras and H-ras mutations in the isoprene-induced tumours of Harderian gland, lung and forestomach were observed in mice after 26-week inhalation exposure to 2200 ml/m$^3$ and a 26-week recovery period without exposure. In the Harderian gland tumours, A→T transversions at K-ras codon 61 (15/30) and C→A transversions at H-ras codon 61 (8/30) (in spontaneous tumours: 2/27 K-ras and 4/25 H-ras) were mainly involved. In the lung tumours, the frequency of A→T transversions in K-ras codon 61 (10/11) (spontaneous tumours: 0/82 K-ras) and in the forestomach the incidence of G→C transversions at K-ras codon 13 (5/10) and A→T transversions at H-ras codon 61 (2/10) (in spontaneous tumours: 1/11 K-ras and 0/11 H-ras) was increased. The activation of K-ras or H-

ras is an important and early step in forming Harderian gland, lung and forestomach tumours. Ras mutations and promoting mechanisms contribute to the process of tumour formation (Hong et al. 1997; Sills et al. 1999 a, b, 2001).

No micronuclei were induced in lung fibroblasts in male and female rats after inhalation of isoprene up to 7000 ml/m$^3$ for four weeks (NTP 1999). On the other hand, in male mice, isoprene produced an increased number of micronuclei-containing erythrocytes after inhalation exposure at 700 ml/m$^3$ and above for 12 days in the peripheral blood (NTP 1999; Placke et al. 1996; Shelby 1990; Shelby and Witt 1995; Tice 1988; Tice et al. 1988).

No induction of chromosome aberration in the bone marrow was observed in male mice after inhaling up to 7000 ml isoprene/m$^3$ for 12 days (Shelby 1990; Shelby and Witt 1995; Tice 1988; Tice et al. 1988).

## 5.7 Carcinogenicity

At present, isoprene is classified by the IARC as being "possibly carcinogenic to humans (Group 2B)" (IARC 1994, 1999).

### 5.7.1 Short term tests

There are no data available for short-term tests with isoprene.

### 5.7.2 Long-term studies

The results of inhalation studies on the carcinogenicity of isoprene in rats and mice are given in Table 11.

**Rat**

In an NTP study, male rats were whole-body exposed to isoprene at 0, 70, 220, 700, 2200 or 7000 ml/m$^3$ for 26 weeks. Already after 26 weeks of exposure, an increase in the incidence of testicular interstitial cell hyperplasia was found in the animals treated with 220, 2200 and 7000 ml/m$^3$. The authors evaluated this as substance-related due to its early occurrence. This effect was statistically significant with an increase in severity in the rats of the highest concentration group. A statistically significant concentration-dependent increase in multiple and single unilateral adenomas of the interstitial cells at 7000 ml/m$^3$ was observed with isoprene after a further 26 exposure-free weeks (compare Section 5.2.1) (Melnick et al. 1992, 1994, 1996; NTP 1995).

**Table 11** Studies on the carcinogenic effects of isoprene: inhalation exposure

| | | | | | | |
|---|---|---|---|---|---|---|
| Author: | Melnick et al. 1992, 1994, 1996; NTP 1995 | | | | | |
| Substance: | Isoprene, purity: > 99% | | | | | |
| Species: | **rat** F344/N, 40 ♂ per group (10 per group for interim sacrifice) | | | | | |
| Administration: | inhalation, whole-body exposure | | | | | |
| Concentration: | 0, 70, 220, 700, 2200, 7000 ml/m$^3$ | | | | | |
| Duration: | 26 wk, 6 h/day, 5 days/wk, recovery period: 26 wk | | | | | |
| Toxicity: | None | | | | | |
| | Isoprene (ml/m$^3$) | | | | | |
| | 0 | 70 | 220 | 700 | 2200 | 7000 |
| **Tumours and preneoplasias** | | | | | | |
| **Testes:** | | | | | | |
| hyperplasia of interstitial cells after 26 weeks | 1/10 (10%) | 1/10 (10%) | 3/10 (30%) | 1/10 (10%) | 3/10 (30%) | 10/10 (100%)** |
| hyperplasia of interstitial cells after 52 weeks | 25/30 (83%) | 30/30 (100%)* | 28/30 (93%) | 30/30 (100%)* | 29/29 (100%)* | 30/30 (100%)* |
| adenomas of interstitial cells, including multiple adenomas | 3/30 (10%) | 3/30 (10%) | 4/30 (13%) | 7/30 (23%) | 8/29 (28%) | 9/30 (30%)*** |

* $p < 0.05$; ** $p < 0.01$ (Fisher's exact test); *** $p = 0.021$ (Cochran-Armitage trend test); incidences from NTP (1995) partly higher than given in Melnick et al. (1994)

| | | | | | |
|---|---|---|---|---|---|
| Author: | NTP 1999 | | | | |
| Substance: | Isoprene, purity: > 99% | | | | |
| Species: | **rat** F344/N, 50 ♂/♀ per group | | | | |
| Application: | inhalation, whole-body exposure | | | | |
| Concentration: | 0, 220, 700, 7000 ml/m$^3$ | | | | |
| Duration: | 105 wk, 6 h/day, 5 days/wk | | | | |
| Toxicity: | **at 700 ml/m$^3$ and above**: ♂: splenic fibrosis, renal tubular hyperplasia;<br>**7000 ml/m$^3$**: ♂: hyperplasia in the parathyroid gland;<br>♀: suppurative inflammation in the nose, bile duct hyperplasia | | | | |
| | | Isoprene (ml/m$^3$) | | | |
| | | 0 | 220 | 700 | 7000 |
| Survivors after 24 months | ♂ | 18/50 | 16/50 | 15/50 | 15/50 |
| | ♀ | 29/50 | 30/50 | 28/50 | 27/50 |

**Table 11** (Continued)

| **Tumours and preneoplastic lesions** | | | | | |
|---|---|---|---|---|---|
| **Kidney:** | | | | | |
| renal tubule, hyperplasia (standard evaluation)[1)] | ♂ | 0/50 (0%) | 2/50 (4%) | 6/50 (12%)* | 8/50 (16%)** |
| renal tubule, hyperplasia (standard and extended evaluation)[1)] | ♂ | 7/50(14%) | 6/50(12%) | 13/50 (26%) | 18/50 (36%)** |
| renal tubule adenoma (standard evaluation)[1)] | ♂ | 0/50 (0%) | 2/50 (4%) | 2/50 (4%) | 6/50 (12%)* |
| renal tubule adenoma (standard and extended evaluation)[1)] | ♂ | 2/50 (4%) | 4/50 (8%) | 8/50 (16%)* | 15/50 (30%)** |
| **Mammary gland:** | | | | | |
| fibroadenoma, multiple | ♂ | 1/50 (2%) | 1/50 (2%) | 0/50 (0%) | 7/50 (14%)* |
| | ♀ | 7/50 (14%) | 12/50 (24%) | 19/50 (38%)** | 17/50 (34%)** |
| fibroadenoma, multiple and single | ♂[2)] | 2/50 (4%) | 4/50 (8%) | 6/50 (12%) | 21/50 (42%)** |
| | ♀[2)] | 19/50 (38%) | 35/50 (70%)** | 32/50 (64%)** | 32/50 (64%)** |
| carcinoma | ♂[2)] | 0/42 (0%) | 1/43 (2%) | 1/47 (2%) | 2/44 (5%) |
| | ♀ | 4/50 (8%) | 2/50 (4%) | 1/50 (2%) | 3/50 (6%) |
| **Testes:** | | | | | |
| interstitial cell adenoma, bilateral | ♂ | 20/50 (40%) | 29/50 (58%) | 37/50 (74%)** | 48/50 (96%)** |
| interstitial cell adenoma, unilateral and bilateral | ♂[3)] | 33/50 (66%) | 37/50 (74%) | 44/50 (88%)* | 48/50 (96%)** |
| **Nervous system/brain:** | | | | | |
| benign astrocytoma | ♀[4)] | 0/50 (0%) | 0/50 (0%) | 1/50 (2%) | 0/50 (0%) |
| malignant astrocytoma | ♂[5)] | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) | 1/50 (2%) |
| malignant glioma | ♀[6)] | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) | 1/50 (2%) |
| malignant medulloblastoma | ♀[7)] | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) | 1/50 (2%) |
| meninges, benign granular cell tumour | ♂[8)] | 0/50 (0%) | 0/50 (0%) | 1/50 (2%) | 0/50 (0%) |
| | ♀[8)] | 0/50 (0%) | 1/50 (2%) | 0/50 (0%) | 1/50(2%) |
| meninges, sarcoma | ♀[9)] | 0/50 (0%) | 1/50(2%) | 0/50 (0%) | 1/50 (2%) |
| **Lymphohaematopoietic system:** | | | | | |
| mononuclear leukaemia | ♀ | 14/50 (28%) | 15/50 (30%) | 21/50 (42%) | 21/50 (42%) |

*p ≤ 0.05;**p ≤ 0.01 (Fisher's exact test)
1)Standard evaluation: one tissue section per kidney, extended evaluation: a number of tissue sections per kidney spaced at 1 mm intervals, i.e. an additional four tissue sections per kidneyHistorical control data of the laboratory: total incidence (average incidence per test ± standard deviation; range)
2)Fibroadenoma ♂: 17/905 (1.9±+2.0%; 0–6%), ♀: 315/903 (34.9±9.9%; 20–54%); carcinoma of the mammary gland ♂ : 1/905 (0.1 ±0.5%; 0–2%);
3)Testicular tumours ♂: 628/905 (69.4±9.7%; 46–83%);
4)Benign astrocytoma ♀: 1/899 (0.1 ±0.5%; 0–2%);
5)Malignant astrocytoma ♂: 1/904 (0.1 ±0.5%; 0–2%);
6)Malignant glioma ♀: 1/899 (0.1 ±0.5%; 0–2%);
7)Malignant medulloblastoma ♀: 0/899;
8)Benign granular cell tumour of the meninges ♂: 0/904; ♀ : 2/899 (0.2±0.7%; 0–2%);
9)Sarcoma of the meninges ♀: 0/899

**Table 11** (Continued)

| | |
|---|---|
| Author: | Melnick et al. 1992, 1994, 1996; NTP 1995 |
| Substance: | Isoprene, purity: > 99% |
| Species: | **mouse** B6C3F$_1$, 40 ♂ per group (10 per group for interim sacrifice) |
| Administration: | inhalation, whole-body exposure |
| Concentration: | 0, 70, 220, 700, 2200, 7000 ml/m$^3$ |
| Duration: | 26 wk, 6 h/day, 5 days/wk, recovery period: 26 wk |
| Toxicity: | **After 26 weeks:**<br>**at 220 ml/m$^3$ and above:** grip strength of fore- and hindlimbs decreased<br>**at 700 ml/m$^3$ and above:** epithelial hyperplasia of the forestomach, macrocytic anaemia;<br>**7000 ml/m$^3$:** degeneration of the olfactory epithelium in the<br>turbinates, spinal cord degeneration, atrophy of skeletal muscles, testicular atrophy<br>**after 52 weeks:**<br>**at 70 ml/m$^3$ and above:** spinal cord degeneration ;<br>**at 700 ml/m$^3$ and above:** degeneration of the olfactory epithelium in the turbinates, epithelial hyperplasia of the forestomach;<br>**at 2200 ml/m$^3$ and above:** hyperplasia of the alveolar epithelium;<br>**7000 ml/m$^3$:** mortality increased, testicular atrophy |

| | Isoprene (ml/m$^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 70 | 220 | 700 | 2200 | 7000 |
| Survivors after 26 weeks | 39/40 | 39/40 | 40/40 | 39/40 | 38/40 | 34/40 |
| Survivors after 52 weeks | 27/30 | 28/30 | 28/30 | 27/30 | 26/30 | 21/30$^{*}$ |
| **Tumours and preneoplastic lesions** | | | | | | |
| **Harderian gland:** | | | | | | |
| hyperplasia | 1/30 (3%) | 0/30 (0%) | 2/29 (7%) | 2/30 (7%) | 2/30 (7%) | 2/28 (7%) |
| adenoma | 2/30 (7%) | 6/30 (20%) | 4/30 (13%) | 14/30 (47%)$^{***}$ | 13/30[1)] (43%)$^{***}$ | 12/30 (40%)$^{***}$ |
| **Liver:** | | | | | | |
| basophilic foci | 3/30 (10%) | 1/30 (3%) | 1/29 (3%) | 2/30 (7%) | 5/30 (17%) | 3/28 (11%) |
| eosinophilic foci | 1/30 (3%) | 0/30 (0%) | 0/29 (0%) | 6/30 (20%) | 5/30 (17%) | 3/28 (11%) |
| mixed foci | 0/30 (0%) | 0/30 (0%) | 1/29 (3%) | 1/30 (3%) | 2/30 (7%) | 3/28 (11%) |
| hepatocellular adenoma | 4/30 (13%) | 2/30 (7%) | 6/29 (21%) | 15/30 (50%)$^{***}$ | 18/30 (60%)$^{***}$ | 16/28 (57%)$^{***}$ |
| hepatocellular carcinoma | 4/30 (13%) | 1/30 (3%) | 3/29 (10%) | 5/30 (17%) | 4/30 (13%) | 9/28 (32%)$^{*}$ |
| hepatocellular adenoma and carcinoma | 7/30 (23%) | 3/30 (10%) | 7/29 (24%) | 15/30 (50%)$^{*}$ | 18/30 (60%)$^{***}$ | 17/28 (61%)$^{***}$ |

**Table 11** (Continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| **Lung:** | | | | | | |
| hyperplasia of the alveolar epithelium | 0/30 (0%) | 1/30 (3%) | 0/29 (0%) | 3/30 (10%) | 4/30 (13%) | 7/28 (25%)*** |
| alveolar/bronchiolar adenoma | 2/30 (7%) | 2/30 (7%) | 1/29 (3%) | 4/30 (13%) | 10/30 (33%)* | 8/28 (29%)* |
| alveolar/bronchiolar carcinoma | 0/30 (0%) | 0/30 (0%) | 0/29 (0%) | 1/30 (3%) | 1/30 (3%) | 3/28 (11%) |
| alveolar/bronchiolar adenoma and carcinoma | 2/30 (7%) | 2/30 (7%) | 1/29 (3%) | 5/30 (17%) | 10/30 (33%)* | 9/28 (32%)*** |
| **Forestomach:** | | | | | | |
| hyperplasia of the epithelium | 1/30 (3%) | 2/30 (7%) | 0/29 (0%) | 8/30 (27%)* | 9/30 (30%)*** | 6/28 (21%) |
| squamous cell papilloma | 0/30 (0%) | 0/30 (0%) | 0/30 (0%) | 1/30 (3%) | 2/30 (7%) | 5/30 (17%) |
| squamous cell carcinoma | 0/30 (0%) | 0/30 (0%) | 0/30 (0%) | 0/30 (0%) | 2/30 (7%) | 1/30 (3%) |
| squamous cell papilloma and carcinoma | 0/30 (0%) | 0/30 (0%) | 0/30 (0%) | 1/30 (3%) | 4/30 (13%) | 6/30 (20%)* |

* $p < 0.05$ (logistic regression test);** $p < 0.01$ (Fisher's exact test);*** $p < 0.01$ (logistic regression test)
ij one mouse had an additional carcinoma

| | |
|---|---|
| Author: | Cox et al. 1996; Placke et al. 1996 |
| Substance: | Isoprene ≥ 99%, <1% limonene (tert-butyl catechol as stabilizer (concentration: 50 ppm) |
| Species: | **mouse** B6C3F$_1$, 50 ♂ per group |
| Administration: | inhalation; whole-body exposure |
| Concentration: | 0, 10, 70, 140, 280, 700, 2200 ml/m$^3$ |
| Duration: | 8 h/day, 5 days/wk<br>20 w: 0, 280, 2200 ml/m$^3$; (2200 ml/m$^3$, 4 h)<br>40 w: 0, 70, 140, 2200 ml/m$^3$<br>80 w: 0, 10, 70, 280, 700, 2200 ml/m$^3$ (2200 ml/m$^3$, 4 and 8 h)<br>recovery period: up to week 104 each |
| Toxicity: | **at 10 ml/m$^3$ and above:** proliferation of haematopoietic cells in the spleen,<br>myeloid hyperplasia of the bone marrow;<br>**at 280 ml/m$^3$ and above:** survival rate after 80 weeks exposure < 50%<br>(no further details), mild metaplasia of the olfactory epithelium to respiratory epithelium;<br>**at higher concentrations** (no further details): hyperplasia of the alveolar epithelium, focal areas of epithelial hyperplasia of the forestomach mucosa, chronic degeneration of the myocardial muscle in the region of the interventricular septum, seminiferous cell atrophy, sperm granulomas |

**Table 11** (Continued)

| Tumours after 104 weeks: | | | | | | | |
|---|---|---|---|---|---|---|---|
| **80-week exposure** | | | | | | | |
| | Isoprene (ml/m$^3$) | | | | | | |
| | 0 | 10 | 70 | 280 | 700 | 2200 (4h) | 2200 (8h) |
| | Cumulative exposure (ml/m$^3$ × weeks) | | | | | | |
| | 0 | 800 | 5600 | 22 400 | 56 000 | 88 000 | 176 240 |
| **Harderian gland:** | | | | | | | |
| adenoma | 4/47 (8%) | 4/49 (8%) | 9/50 (18%) | 17/50 (34%)** | 26/49 (52%)** | 28/50 (56%)** | 35/50 (70%)** |
| carcinoma | 0/47 (0%) | 0/49 (0%) | 0/50 (0%) | 1/50 (2%) | 3/49 (6%) | 2/50 (4%) | 2/50 (4%) |
| **Liver:** | | | | | | | |
| hepatocellular adenoma | 11/50 (22%) | 12/50 (24%) | 15/50 (30%) | 24/50 (48%)** | 27/48 (56%)** | 21/50 (42%)** | 30/50 (60%)** |
| hepatocellular carcinoma | 9/50 (18%) | 6/50 (12%) | 9/50 (18%) | 16/50 (32%) | 17/48 (35%)* | 15/50 (30%) | 16/50 (32%) |
| **Lung:** | | | | | | | |
| alveolar/bronchiolar adenoma | 11/50 (22%) | 16/50 (32%) | 4/50 (8%) | 13/50 (26%) | 23/50 (46%)** | 15/50 (30%) | 30/50 (60%)** |
| alveolar/bronchiolar carcinoma | 0/50 (0%) | 1/50 (2%) | 2/50 (4%) | 1/50 (2%) | 7/50 (14%)** | 3/50 (6%) | 7/50 (14%)** |
| **Spleen:** | | | | | | | |
| haemangiosarcoma | 1/49 (2%) | 3/48 (6%) | 2/50 (4%) | 1/50 (2%) | 2/48 (4%) | 2/50 (4%) | 1/49 (2%) |
| **Heart:** | | | | | | | |
| haemangiosarcoma | 0/49 (0%) | 0/50 (0%) | 0/50 (0%) | 2/50 (4%) | 1/50 (2%) | 1/50 (2%) | 1/50 (2%) |
| **Forestomach:** | | | | | | | |
| squamous cell papilloma | 0/50 (0%) | 0/48 (0%) | 0/50 (0%) | 0/50 (0%) | 1/47 (2%) | 1/50 (2%) | 1/50 (2%) |
| squamous cell carcinoma | 0/50 (0%) | 0/48 (0%) | 0/50 (0%) | 1/50 (2%) | 0/47 (0%) | 1/50 (2%) | 3/50 (6%) |
| **Lymphohaematopoietic system:** | | | | | | | |
| histiocytic sarcoma | 0/50 (0%) | 2/50 (4%) | 2/50 (4%) | 4/50 (8%) | 2/50 (4%) | 7/50 (14%)** | 2/50 (4%) |
| lymphoma | 2/50 (4%) | 1/50 (2%) | 4/50 (8%) | 5/50 (10%) | 4/50 (8%) | 4/50 (8%) | 6/50 (12%) |

| **40-week exposure** | | | | |
|---|---|---|---|---|
| | Isoprene (ml/m$^3$) | | | |
| | 0 | 70 | 140 | 2200 |
| | Cumulative exposure (ml/m$^3$ × weeks) | | | |
| | 0 | 2800 | 5600 | 88 000 |
| **Harderian gland:** | | | | |
| adenoma | 4/47 (9%) | 13/48 (27%)* | 12/50 (24%)* | 31/49 (63%)** |
| carcinoma | 0/47 (0%) | 0/48 (0%) | 2/50 (4%) | 0/49 (0%) |

**Table 11** (Continued)

| | | | | |
|---|---|---|---|---|
| **Liver:** | | | | |
| hepatocellular adenoma | 11/50 (22%) | 14/49 (29%) | 22/50 (44%)* | 28/47 (60%)** |
| hepatocellular carcinoma | 9/50 (18%) | 11/49 (23%) | 10/50 (20%) | 18/47(38%)* |
| **Lung:** | | | | |
| alveolare/bronchiolar adenoma | 11/50 (22%) | 8/50 (16%) | 10/50 (20%) | 29/49 (59%)** |
| alveolare/bronchiolar carcinoma | 0/50 (0%) | 0/50 (0%) | 1/50 (2%) | 3/49 (6%) |
| **Spleen:** | | | | |
| haemangiosarcoma | 1/49 (2%) | 1/47 (2%) | 3/50 (6%) | 0/47 (0%) |
| **Heart:** | | | | |
| haemangiosarcoma | 0/49 (0%) | 0/49 (0%) | 0/50 (0%) | 1/49 (2%) |
| **Forestomach:** | | | | |
| squamous cell papilloma | 0/50 (0%) | 0/47 (0%) | 0/49 (0%) | 2/47 (4%) |
| **Lymphohaematopoietic system:** | | | | |
| histiocytic sarcoma | 0/50 (0%) | 2/50 (4%) | 1/50 (2%) | 7/50 (14%)** |
| lymphoma | 2/50 (4%) | 2/50 (4%) | 1/50 (2%) | 5/50 (10%) |
| **20-week exposure** | | | | |
| | | Isoprene (ml/m$^3$) | | |
| | | 0 | 280 | 2200 (4 h) |
| | | Cumulative exposure (ml/m$^3$ × weeks) | | |
| | | 0 | 5600 | 22 000 |
| **Harderian gland:** | | | | |
| adenoma | | 4/47 (9%) | 16/49 (32%)** | 19/49 (40%)** |
| carcinoma | | 0/47 (0%) | 3/49 (6%) | 1/49 (2%) |
| **Liver:** | | | | |
| hepatocellular adenoma | | 11/50 (22%) | 18/49 (37%) | 22/50 (44%)* |
| hepatocellular carcinoma | | 9/50 (18%) | 12/49 (25%) | 12/50 (24%) |
| **Lung:** | | | | |
| alveolar/bronchiolar adenoma | | 11/50 (22%) | 16/50 (32%) | 14/50 (28%) |
| alveolar/bronchiolar carcinoma | | 0/50 (0%) | 3/50 (6%) | 2/50 (4%) |
| **Spleen:** | | | | |
| haemangiosarcoma | | 1/49 (2%) | 2/47 (4%) | 2/48 (4%) |
| **Heart:** | | | | |
| haemangiosarcoma | | 0/49 (0%) | 0/49 (0%) | 4/50 (8%) |
| **Forestomach:** | | | | |
| squamous cell carcinoma | | 0/50 (0%) | 0/46 (0%) | 1/50 (2%) |
| **Lymphohaematopoietic system:** | | | | |
| histiocytic sarcoma | | 0/50 (0%) | 8/50 (16%)** | 5/50 (10%)* |
| lymphoma | | 2/50 (4%) | 7/50 (14%) | 4/50 (8%) |

* $p \leq 0.05$; ** $p \leq 0.01$ (Fisher's exact test), newly calculated

**Table 11** (Continued)

| | | | |
|---|---|---|---|
| Author: | Cox et al. 1996; Placke et al. 1996 | | |
| Substance: | isoprene ≥ 99%, < 1% limonene, tert-butyl catechol as stabilizer (concentration: 50 ppm) | | |
| Species: | mouse B6C3F$_1$, 50 ♀ per group | | |
| Administration: | inhalation, whole-body exposure | | |
| Concentration: | 0, 10, 70 ml/m$^3$ | | |
| Duration: | 80 wk, 8 h/day, 5 days/wk, recovery period: 24 wk | | |
| Toxicity: | **at 10 ml/m$^3$ and above**: proliferation of haematopoietic cells in the spleen, myeloid hyperplasia of the bone marrow<br>**70 ml/m$^3$:** mild metaplasia of the olfactory epithelium to respiratory epithelium | | |
| **Tumours after 104 weeks**: | | | |
| | Isoprene (ml/m$^3$) | | |
| | 0 | 10 | 70 |
| | Cumulative exposure (ml/m$^3$ × weeks) | | |
| | 0 | 800 | 5600 |
| **Harderian gland:** | | | |
| adenoma | 2/49 (4%) | 3/49 (6%) | 8/49(16%)* |
| **Spleen:** | | | |
| haemangiosarcoma | 1/50 (2%) | 1/49 (2%) | 4/50 (8%) |
| **Pituitary gland:** | | | |
| adenoma | 1/49 (2%) | 6/46 (13%) | 9/49 (18%)** |
| **Lymphohaematopoietic system:** | | | |
| histiocytic sarcoma | 4/50 (8%) | 5/50 (10%) | 6/50 (12%) |
| lymphoma | 9/50 (18%) | 10/50 (20%) | 12/50 (24%) |

*p ≤ 0.05; **p ≤ 0.01 (Fisher's exact test), newly calculated

In an NTP carcinogenicity study, male and female F344/N rats inhaled isoprene at concentrations of 0, 220, 700 or 7000 ml/m$^3$ (whole-body exposure) for 105 weeks. The incidences of mammary fibroadenomas in females at 220 ml/m$^3$ and above, of renal tubule adenomas and of interstitial cell tumours of the testes in males at 770 ml/m$^3$ and above were significantly increased. The incidence of mammary fibroadenomas exceeded the range of historical controls in males at 220 ml/m$^3$ and above. The increase was concentration-dependent, and was significant at 7000 ml/m$^3$. These tumours were assessed as substance-related by the authors. The brain tumours in the exposed female rats (single occurrences of malignant astrocytoma, malignant glioma, malignant medulloblastoma, benign granular cell tumour and sarcoma of the meninges in one animal each of the high concentration group) were regarded as potentially substance-related since these tumours occur rarely.

**Table 11** (Continued)

Survival, body weight and clinical signs were unaffected by isoprene exposure (see Section 5.2.1) (NTP 1999). The LOAEC is thus 220 ml/m$^3$.

### Mouse

In an NTP study, male B6C3F$_1$ mice were whole-body exposed to 0, 70, 220, 700, 2200 or 7000 ml isoprene/m$^3$ for 26 weeks. They were then monitored for a further 26 weeks. In the male mice treated, the incidences of the following types of tumour were increased by the end of the recovery period: Harderian gland adenomas (numerical increase at 70 ml/m$^3$ and above, statistically significant increase at 700 ml/m$^3$ and above), hepatocellular adenomas and carcinomas (at 700 ml/m$^3$ and above), alveolar/bronchiolar adenomas and carcinomas (at 2200 ml/m$^3$ and above) and squamous cell papillomas and carcinomas of the forestomach (increase numerical at 2200 ml/m$^3$ and above, statistically significant at 7000 ml/m$^3$). Mortality was increased by the end of the study at 2200 and 7000 ml/m$^3$ (see Section 5.2.1, Section 5.5.1) (Melnick et al. 1992, 1994, 1996; NTP 1995).

It was the purpose of a study with male and female B6C3F$_1$ mice to investigate the influence of concentration and exposure duration on tumour incidences from isoprene. In the males, isoprene produced significantly increased incidences of Harderian gland adenomas (at 70 ml/m$^3$ and above), hepatocellular adenomas (at 140 ml/m$^3$ and above) and carcinomas (at 700 ml/m$^3$ and above), alveolar/bronchiolar adenomas and carcinomas (700 ml/m$^3$) and histiocytic sarcomas (280 ml/m$^3$). Harderian gland adenomas already occurred in the animals exposed for only 20 weeks. Haemangiosarcomas in the heart (at 280 ml/m$^3$ and above) were observed in exposed male mice only, but were not statistically significant. This type of haemangiosarcoma is extremely rare in B6C3F$_1$ mice: in fact, none were found in 658 historical controls. In female mice, the incidence of haemangiosarcomas in the spleen (70 ml/m$^3$) showed a slight but statistically non-significant increase compared with controls. The authors question whether the neoplasms in the Harderian gland or the pituitary gland of females to be substance-related at 70 ml/m$^3$ on account of the historical control data (adenomas of the Harderian gland: 22/661; 3.3%; 0–16%; pituitary gland adenomas: 127/627; 20.2%; 2–44%). In another study, however, mutations in the K-ras gene in tumours of the Harderian gland were demonstrated to be caused by isoprene exposure. Furthermore, this tumour also occurred in male mice and is thus considered to be substance-related. Although the incidence of pituitary gland tumours was significantly increased at 70 ml/m$^3$, it was lower than the average incidence of historical controls. No pituitary gland tumours were found in the males exposed to considerably higher levels. This means that the pituitary gland tumours in the females are not clearly substance-related. The authors conclude that the level of exposure has a greater influence on tumour frequency than its duration. Furthermore, the same concentration/time product produced different tumour frequencies; this was independent of whether daily or weekly exposure duration varied. In addition, a significantly increased tumour incidence was found in the 2200 ml/m$^3$ group

compared with the 280 ml/m$^3$ group (an about 8-fold concentration difference), but not in the 10 ml/m$^3$ group compared with the 70 ml/m$^3$ group (7-fold concentration difference). This argues in favour of a non-linear concentration/tumour frequency relationship. The authors came to the conclusion that tumour risks for isoprene cannot be extrapolated using concentration/time products, so that a complex dynamic risk assessment model is more suitable in this case (Cox et al. 1996; Placke et al. 1996).

### 5.8 Other effects

The inhibitory effect of isoprene on tumour formation was investigated in an initiation-promotion experiment using dimethyl benz[a]anthracene as initiator and croton resin as promotor on the skin of female ICR Swiss mice. Dimethylbenz[a]anthracene (0.125 mg in 0.25 ml acetone) was applied once non-occlusively onto the shaved intact skin of the back to 30 animals. Following a 3-week treatment interval, the shaved skin of the mice was treated with 0.006% croton resin and 0.015% isoprene in acetone 5 days a week for 18 weeks. Corresponding groups received dimethyl benz[a]anthracene plus croton resin as positive controls, or dimethyl benz [a]anthracene plus acetone or only acetone as negative controls. Tumours developed in 90% of the animals treated with croton resin and isoprene and 90% of the positive controls. In contrast, no neoplasms occurred in the animals of both negative controls. There was a slight reduction in the number of papillomas per mouse when comparing the positive controls (12.2 papillomas) with those having received isoprene and croton resin (8 papillomas, no data on statistical significance) (Shamberger 1971).

Groups of 5–7 male Swiss mice were administered isoprene (no data on mode of administration) at a dose level of 500 mg/kg body weight daily for two or seven days. The cytochrome $b_5$ and cytochrome P-450 levels and the activities of aminopyrine N-demethylase, dinemorphan N-demethylase and isoprene epoxidase were unchanged compared with controls (Del Monte et al. 1985).

## 6 Manifesto

After inhalation at 70 ml/m$^3$ and above, isoprene produced adenomas of the Harderian gland (Cox et al. 1996; Placke et al. 1996) and, at higher concentrations, tumours in liver, lungs and forestomach, and haemangiosarcomas and histiocytic sarcomas in mice (Cox et al. 1996; NTP 1995; Placke et al. 1996). At 10 ml/m$^3$, no significantly increased tumour incidence was recorded. After isoprene inhalation exposure, at the lowest concentration tested of 220 ml/m$^3$ and above, significantly increased incidences of tumours in the mammary gland, the kidneys and the interstitial cells of the testes in rats were observed (NTP 1999). The demonstrated

genotoxicity is considered to be the cause of the tumours. As a MAK value can be derived for isoprene (see below), and the carcinogenic and genotoxic effects are considered to be so low that a significant contribution to the cancer risk in humans is not to be expected when the MAK value is observed, isoprene is classified in Carcinogen Category 5.

In bacterial test systems, isoprene itself has no mutagenic effects, but only the diepoxide methyl-1,2:3,4-diepoxybutane. Derivation of a MAK value from internal exposure to mutagenic metabolites is not possible, as the required in vitro and in vivo measurements are not available. A meaningful exposure parameter such as the area under the concentration-time curve in the blood (AUC) can, however, be given for isoprene itself. It is formed endogenously in humans. The AUC after exposure to an isoprene concentration of about 3 ml/m$^3$ for eight hours daily over 40 years is the same as the AUC for lifelong exposure at the level of the standard deviation of the mean endogenous isoprene concentration (Section 3.6). Therefore, exposure to 3 ml/m$^3$ makes no significant contribution to the cancer risk. A MAK value of 3 ml/m$^3$ is established for isoprene. As systemic effects are the main effects, isoprene is assigned to peak limitation category II. An excursion factor of 8 has been established, as only the AUC and not the concentration are decisive for the effects due to the assumed genotoxic mechanism of action.

There are no genotoxicity tests with isoprene in germ cells. After inhalation, isoprene induced micronuclei in erythrocytes and increased SCE frequencies in the bone marrow of mice, with an NOAEC of 70 ml/m$^3$. The germ cells are reached, since after inhalation in mice and rats effects on the sperms and/or the testes were observed at 700 ml/m$^3$, but not at 70 ml/m$^3$ (see Section 5.5.1). With isoprene, therefore, a germ cell mutagenic effect is suspected. But as neither a mutagenic effect in soma cells nor a toxic effect in germ cells is observed at 70 ml/m$^3$, the potency of which is considered to be so low that no significant contribution to a genetic risk in humans is to be expected provided the MAK value is observed. Therefore, isoprene is classified in Category 5 for Germ Cell Mutagens.

No data on the dermal penetration of liquid or gaseous isoprene are available. Though model calculations indicate that the substance has properties facilitating absorption through the skin, these are only to be used with a certain reserve when making a realistic estimation due to the extreme volatility of the substance. In the case of hydrocarbons, dermal absorption from the gas phase is, compared with their inhalation absorption, low (McDougal et al. 1990). This means that, when observing the MAK value, no marked increase in the physiological isoprene levels result from the dermally absorbed quantities. Therefore, isoprene is not designated with "H".

No animal studies or studies in humans are available on skin and respiratory sensitization. Therefore, no such effects are suspected and designation with an "Sh" or "Sa" is not necessary.

In rats, the NOAEC for developmental toxicity with isoprene was 7000 ml/m$^3$ (highest tested concentration), and in mice 280 ml/m$^3$ (decreased foetal weight at

1400 ml/m³ and above) (NTP 1995). The difference to the MAK value of 3 ml/m³ is sufficiently great, so that isoprene is classified in Pregnancy Risk Group C.

## References


Barker M, Hengst M, Schmid J, Buers H-J, Mittermaier B, Klemp D, Koppmann R (2006) Volatile organic compounds in the exhaled breath of young patients with cystic fibrosis. *Eur Respir J* 27: 929–936

Begemann P, Christova-Georgieva NI, Sangaiah R, Koc H, Zhang D, Golding BT, Gold A, Swenberg JA (2004) Synthesis, characterization, and identification of N7-guanine adducts of isoprene monoepoxides *in vitro*. *Chem Res Toxicol* 17: 929–936

BG Chemie (Berufsgenossenschaft der chemischen Industrie / Chemical Industry Employers' Liability Insurance Association) (2000) TOXIKOLOGISCHE BEWERTUNG [Existing Substances Evaluation] No. 105, Isoprene, BG Chemie, Heidelberg (German), www.bgchemie.de/files/95/ToxBew105-L.pdf

Bleasdale C, Small RD, Watson WP, Wilson J, Golding BT (1996) Studies on the molecular toxicology of buta-1,3-diene and isoprene epoxides. *Toxicology* 113: 290–293

Bogaards JJP, Venekamp JC, van Bladeren PJ (1996) The biotransformation of isoprene and the two isoprene monoepoxides by human cytochrome P450 enzymes, compared to mouse and rat liver microsomes. *Chem Biol Interact* 102: 169–182

Bogaards JJP, Venekamp JC, Salmon FGC, van Bladeren PJ (1999) Conjugation of isoprene monoepoxides with glutathione, catalyzed by α, μ, μ and θ-class glutathione S-transferases of rat and man. *Chem Biol Interact* 117: 1–14

Bogaards JJ, Freidig AP, van Bladeren PJ (2001) Prediction of isoprene diepoxide levels *in vivo* in mouse, rat and man using enzyme kinetic data *in vitro* and physiologically-based pharmacokinetic modelling. *Chem Biol Interact* 138: 247–265

Bond JA, Bechtold WE, Birnbaum LS, Dahl AR, Medinsky MA, Sun JD, Henderson RF (1991) Disposition of inhaled isoprene in B6C3$F_1$ mice. *Toxicol Appl Pharmacol* 107: 494–503

Buckley LA, Coleman DP, Burgess JP, Thomas BF, Burka LT, Jeffcoat AR (1999) Identification of urinary metabolites of isoprene in rats and comparison with mouse urinary metabolites. *Drug Metab Dispos* 27: 848–854

Cailleux A, Allain P (1989) Isoprene and sleep. *Life Sci* 44: 1877–1880

Cailleux A, Cogny M, Allain P (1992) Blood isoprene concentrations in humans and in some animal species. *Biochem Med Metab Biol* 47: 157–160

CambridgeSoft (2006) Chemfinder database, http://chemfinder.cambridgesoft.com/result.asp?molid=78-79-5

Chiappe C, De Rubertis A, Tinagli V, Amato G, Gervasi PG (2000) Stereochemical course of the biotransformation of isoprene monoepoxides and of the corresponding diols with liver microsomes from control and induced rats. *Chem Res Toxicol* 13: 831–838

Conkle JP, Camp BJ, Welch BE (1975) Trace composition of human respiratory gas. *Arch Environ Health* 30: 290–295

Cope KA, Watson MT, Foster WM, Sehnen SS, Risby TH (2004) Effects of ventilation on the collection of exhaled breath in humans. *J Appl Physiol* 96: 1371–1379

Cox LA Jr, Bird MG, Griffis L (1996) Isoprene cancer risk and the time pattern of dose administration. *Toxicology* 113: 263–272

Csanády GA, Filser JG (2001 a) The relevance of physical activity for the kinetics of inhaled gaseous substances. *Arch Toxicol* 74: 663–672

Csanády GA, Filser JG (2001 b) Toxicokinetics of inhaled and endogenous isoprene in mice, rats, and humans. *Chem Biol Interact* 135–136: 679–685

Dahl AR, Birnbaum LS, Bond JA, Gervasi PG, Henderson RF (1987) The fate of isoprene inhaled by rats: comparison to butadiene. *Toxicol Appl Pharmacol* 89: 237–248

Dahl AR, Bechtold WE, Bond JA, Henderson RF, Mauderly JL, Muggenburg BA, Sun JD, Birnbaum LS (1990) Species differences in the metabolism and disposition of inhaled 1,3-butadiene and isoprene. *Environ Health Perspect* 86: 65–69

Davies S, Španěl P, Smith D (2001) A new 'online' method to measure increased exhaled isoprene in end-stage renal failure. *Nephrol Dial Transplant* 16: 836–839

DeMaster EG, Nagasawa HT (1978) Isoprene, an endogenous constituent of human alveolar air with a diurnal pattern of excretion. *Life Sci* 22: 91–98

Del Monte M, Citti L, Gervasi PG (1985) Isoprene metabolism by liver microsomal monooxygenases. *Xenobiotica* 15: 591–597

Deneris ES, Stein RA, Mead JF (1984) In vitro biosynthesis of isoprene from mevalonate utilizing a rat liver cytosolic fraction. *Biochem Biophys Res Commun* 123: 691–696

Deneris ES, Stein RA, Mead JF (1985) Acid-catalyzed formation of isoprene from mevalonate-derived product using a rat liver cytosolic fraction. *J Biol Chem* 260: 1382–1385

Diskin AM, Španěl P, Smith D (2003) Time variation of ammonia, acetone, isoprene and ethanol in breath: a quantitative SIFT-MS study over 30 days. *Physiol Meas* 24: 107–119

Doerr JK, Hooser SB, Smith BJ, Sipes IG (1995) Ovarian toxicity of 4-vinylcyclohexene and related olefins in $B6C3F_1$ mice: role of diepoxides. *Chem Res Toxicol* 8: 963–969

Faustov AO (1972) Toxisch-hygienische Charakteristik des Gasfaktors bei der Herstellung einiger Arten von synthetischen Kautschuken für allgemeine Verwendungszwecke (Toxicohygienic characteristic of the gas factor in the manufacture of several kinds of synthetic rubbers for general uses) (deutsche Übersetzung aus dem Russischen). Tr Voronezh Meditsinskii Inst (German, from the Russian in: Трудовой Воронеж Медицинский Институт) (Russian) 87: 10–16

Fenske JD, Paulson SE (1999) Human breath emission of VOCs. *J Air Waste Manag Assoc* 49: 594–598

Filser JG, Csanády GA, Denk B, Hartmann M, Kauffmann A, Kessler W, Kreuzer PE, Pütz C, Shen JH, Stei P (1996) Toxicokinetics of isoprene in rodents and humans. *Toxicology* 113: 278–287

Fiserova-Bergerova V (Hrsg) (1983) Modeling of inhalation exposure to vapors: uptake, distribution, and elimination, Band II, CRC Press, Boca Raton, FL, USA

Foster WM, Jiang L, Stetkiewicz PT, Risby TH (1996) Breath isoprene: temporal changes in respiratory output after exposure to ozone. *J Appl Physiol* 80: 706–710

Gage JC (1970) The subacute inhalation toxicity of 109 industrial chemicals. *Br J Ind Med* 27: 1–18

Gargas ML, Burgess RJ, Voisard DE, Cason GH, Andersen ME (1989) Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol Appl Pharmacol* 98: 87–99

Gelmont D, Stein RA, Mead JF (1981) Isoprene – the main hydrocarbon in human breath. *Biochem Biophys Res Commun* 99: 1456–1460

Gervasi PG, Citti L, Del Monte M, Longo V, Benetti D (1985) Mutagenicity and chemical reactivity of epoxidic intermediates of the isoprene metabolism and other structurally related compounds. *Mutat Res* 156: 77–82

Golding BT, Cottrell L, Mackay D, Zhang D, Watson WP (2003) Stereochemical and kinetic comparisons of mono- and diepoxide formation in the *in vitro* metabolism of isoprene by liver microsomes from rats, mice, and humans. *Chem Res Toxicol* 16: 933–944

Gostinskii VD (1965) The toxicity of isoprene and the maximum permissible concentration of its vapours in the atmosphere of industrial premises (russ, engl summary). *Gig Tr Prof Zabol* 10: 36–42

Grote C, Pawliszyn J (1997) Solid-phase microextraction for the analysis of human breath. *Anal Chem* 69: 587–596

Guy RH, Potts RO (1993) Penetration of industrial chemicals across the skin: a predictive model. *Am J Ind Med* 23: 711–719

Hansel A, Jordan A, Holzinger R, Prazeller P, Vogel W, Lindinger W (1995) Proton transfer reaction mass spectrometry: on-line trace gas analysis at ppb level. *Int J Mass Spectrom* 149: 609–619

Hong HL, Devereux TR, Melnick RL, Eldridge SR, Greenwell A, Haseman J, Boorman GA, Sills RC (1997) Both K-ras and H-ras protooncogene mutations are associated with Harderian gland tumorigenesis in B6C3F1 mice exposed to isoprene for 26 weeks. *Carcinogenesis* 18: 783–789

Hyšpler R, Crhová Š, Gasparič J, Zadák Z, Cizková M, Balasová V (2000) Determination of isoprene in human expired breath using solid-phase microextraction and gas chromatography-mass spectrometry. *J Chromatogr B* 739: 183–190

IARC (International Agency for Research on Cancer) (1994) Isoprene. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol. 60, IARC, Lyons, FR, 215–232

IARC (1999) Isoprene. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol. 71, IARC, Lyons, FR, 1015–1025

Jansson BO, Larsson BT (1969) Analysis of organic compounds in human breath by gas chromatography-mass spectrometry. *J Lab Clin Med* 74: 961 –966

Jones AW, Lagesson V, Tagesson C (1995) Determination of isoprene in human breath by thermal desorption gas chromatography with ultraviolet detection. *J Chromatogr* 672: 1–6

Karl T, Prazeller P, Mayr D, Jordan A, Rieder J, Fall R, Lindinger W (2001) Human breath isoprene and its relation to blood cholesterol levels: new measurements and modeling. *J Appl Physiol* 91: 762–770

Kohlmüller D, Kochen W (1993) Is n-pentane really an index of lipid peroxidation in humans and animals? A methodological reevaluation. *Anal Biochem* 210: 268–276

Komatsu S (1971) Teratogenic effects of vitamin A. 2. Chemical structure of vitamin A (jpn). *Shikwa Gakuho* 71: 2075–2081

Krotoszynski B, Bruneau GM, O'Neill HJ (1979) Measurement of chemical inhalation exposure in urban population in the presence of endogenous effluents. *J Anal Toxicol* 3: 225–234

Kushi A, Yoshida D, Mizusaki S (1985) Mutagenicity of gaseous nitrogen oxides and olefins on Salmonella TA102 and TA104. *Mutat Res* 147: 263–264

Lärstad M, Loh C, Ljungkvist G, Olin AC, Torén K (2002) Determination of ethane, pentane and isoprene in exhaled air using a multi-bed adsorbent and end-cut gas-solid chromatography. *Analyst* 127: 1440–1445

Lärstad MA, Torén K, Bake B, Olin AC (2007) Determination of ethane, pentane and isoprene in exhaled air – effects of breath-holding, flow rate and purified air. *Acta Physiol (Oxf)* 189: 87–98

Leber AP (2001) Overview of isoprene monomer and polyisoprene production processes. *Chem Biol Interact* 135–136: 169–173

Lechner M, Moser B, Niederseer D, Karlseder A, Holzknecht B, Fuchs M, Colvin S, Tilg H, Rieder J (2006) Gender and age specific differences in exhaled isoprene levels. *Respir Physiol Neurobiol* 154: 478–483

Longo V, Citti L, Gervasi PG (1985) Hepatic microsomal metabolism of isoprene in various rodents. *Toxicol Lett* 29: 33–37

Lynch J (2001) Occupational exposure to butadiene, isoprene and chloroprene. *Chem Biol Interact* 135–136: 207–214

Mamedov AM (1978) Reaktion des Lymphgewebes nach Inhalation von Isopren und einige integrale Indices (Lymphoid tissue reaction and several integral indices following isoprene inhalation: in internet (Medline), English Abstract, Journal Article) (deutsche Übersetzung aus dem Russischen, engl Zusammenfassung) (German, translated from the Russian in: Гигиени Трудовое Профессионалънае Заболевание, Work Hygiene and Professional Medicine). *Gig Tr Prof Zabol* 6: 34–37

Mamedov AM, Aliev VA (1985a) Succinate dehydrogenase activity of immunocompetent cells in workers with occupational exposure in styrene and butadiene rubber production. *Chem Abstr* 102: 296

Mamedov AM, Aliev VA (1985b) Activity of acid and alkaline phosphatases of the blood neutrophils in workers engaged in the manufacture of synthetic rubber (Russian, with English abstract). (Гиг Труд Проф Забол) *Gig Tr Prof Zabol* 5: 31–35

Mast TJ, Rommereim RL, Weigel RJ, Stoney KH, Schwetz BA, Morrissey RE (1990) Inhalation developmental toxicity of isoprene in mice and rats. *Toxicologist* 10: 42 (Abstract).

McDougal JN, Jepson GW, Clewell HK, Gargas ML, Andersen ME (1990) Dermal absorption of organic chemical vapors in rats and humans. *Fundam Appl Toxicol* 14: 299–308

McGrath LT, Patrick R, Silke B (2001) Breath isoprene in patients with heart failure. *Eur J Heart Fail* 3: 423–427

Melnick RL, Roycroft JH, Chou BJ, Ragan HA, Miller RA (1990) Inhalation toxicology of isoprene in F344 rats and B6C3F$_1$ mice following two-week exposures. *Environ Health Perspect* 86: 93–98

Melnick RL, Eustis SL, Chou B, Miller R (1992) Proliferative lesions induced by isoprene in mice, but not in rats, after 26 weeks of inhalation exposure. *Carcinogenesis* 33: 115

Melnick RL, Sills RC, Roycroft JH, Chou BJ, Ragan HA, Miller RA (1994) Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. *Cancer Res* 54: 5333–5339

Melnick RL, Sills RC, Roycroft JH, Chou BJ, Ragan HA, Miller RA (1996) Inhalation toxicity and carcinogenicity of isoprene in rats and mice: comparisons with 1,3-butadiene. *Toxicology* 113: 247–252

Mendis S, Sobotka PA, Euler DE (1994) Pentane and isoprene in expired air from humans: gas-chromatographic analysis of single breath. *Clin Chem* 40: 1485–1488

Mendis S, Sobotka PA, Euler DE (1995) Expired hydrocarbons in patients with acute myocardial infarction. *Free Radic Res* 23: 117–122

de Meester C, Mercier M, Poncelet F (1981) Mutagenic activity of butadiene, hexachlorobutadiene, and isoprene. In: Gut I, Cikrt M, Plaa GL (Eds.) Industrial and environmental xenobiotics, Springer, Berlin, 195–203

Miekisch W, Schubert JK, Vagts DA, Geiger K (2001) Analysis of volatile disease markers in blood. *Clin Chem* 47: 1053–1060

Mitin YV (1969) Über Veränderungen in den oberen Atemwegen von Beschäftigten bei der Herstellung von Isoprenkautschuk (Changes in the upper airways in workers producing isoprene rubber) (deutsche Übersetzung aus dem Russischen) (German, translated from the

Russian). (Журнал Ушн Нос Горл Волез, Journal of Ear, Nose and Throat Diseases) *Zh Ushn Nos Gorl Bolezn* 29: 79–83

Mitsui T, Naitho K, Tsuda T, Hirabayshi T, Kondo T (2000) Is endogenous isoprene the only coeluting compound in the measurement of breath pentane? *Clin Chim Acta* 299: 193 –198

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E (1986) Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ Mutagen* 8, Suppl 7: 1–119

Nelson N, Lagesson V, Nosratabadi AR, Ludvigsson J, Tagesson C (1998) Exhaled isoprene and acetone in newborn infants and in children with diabetes mellitus. *Pediatr Res* 44: 363–367

NTP (National Toxicology Program) (1983) Salmonella mutagenicity test results. NTP Techn Bull 9: 5–6

NTP (1995) Technical report on toxicity studies of isoprene (CAS No. 78-79-5) administered by inhalation to F344/N rats and B6C3F1 mice. NTP Toxicity Report Series No. 31, US Department of Health and Human Services, National Institutes of Health, Bethesda, MD, USA

NTP (1999) Toxicology and carcinogenesis studies of isoprene (CAS No. 78-79-5) in F344/N rats (inhalation studies). NTP Technical Report Series No. 486, US Department of Health and Human Services, National Institutes of Health, Bethesda, MD, USA

OECD (Organisation for Economic Co-operation and Development) (2005) SIDS Initial Assessment Report (SIAR) Isoprene [CAS No 78-79-5]. Final Draft, April 2005, OECD, Paris, http://www.oecd.Org/dataoecd/5/58/35239388.zip

Peter H, Wiegand HJ, Bolt HM, Greim H, Walter G, Berg M, Filser JG (1987) Pharmacokinetics of isoprene in mice and rats. *Toxicol Lett* 36: 9–14

Peter H, Wiegand HJ, Filser JG, Bolt HM, Laib RJ (1990) Inhalation pharmacokinetics of isoprene in rats and mice. *Environ Health Perspect* 86: 89–92

Phillips M, Greenberg J (1991) Method for the collection and analysis of volatile compounds in the breath. *J Chromatogr* 564: 242–249

Placke ME, Griffis L, Bird M, Bus J, Persing RL, Cox LA Jr (1996) Chronic inhalation oncogenicity study of isoprene in $B6C3F_1$ mice. *Toxicology* 110: 253–262

Poli D, Carbognani P, Corradi M, Goldoni M, Acampa O, Balbi B, Bianchi L, Rusca M, Mutti A (2005) Exhaled volatile organic compounds in patients with non-small cell lung cancer: cross sectional and nested short-term study. *Respir Res* 6: 71–80

Repina EF (1988) Untersuchung der selektiven gonadotoxischen Aktivität von Isopren und Piperylen im akuten Versuch (Investigation of selective gonadotoxic activity of isoprene and piperylene in an acute study) (German, translation from the Russian in: Гигиена Производственной и Окрасюсчей Среди, Охрана Здоровья Рабоцих в Нефтехимический Пром-сти, Professional Hygiene in the Painting Branch and Petrochemical Industry) (deutsche Übersetzung aus dem Russischen). Gigiena Proizvodstvennoj i Okruzajuscej Sredy, *Ochrana Zdorov'ja Rabocich v Neftegazodobyv i Neftechim Prom-sti* M: 96–99

Rohr AC, Wilkins CK, Clausen PA, Hammer M, Nielsen GD, Wolkoff P, Spengler JD (2002) Upper airway and pulmonary effects of oxidation products of (+)- α-pinene, d-limonene, and isoprene in BALB/c mice. *Inhal Toxicol* 14: 663–684

Sachs L (1997) Angewandte Statistik (Applied Statistics), Springer, Berlin

Salerno-Kennedy R, Cashman KD (2005) Potential applications of breath isoprene as a biomarker in modern medicine: a concise overview. *Wien Klin Wochenschr* 117: 180–186

Samedov IG, Mamedov AM, Mamedova LN, Bekesev IA (1978) Immunologische Indices als mögliche Kriterien zur Bewertung der Einwirkung von chemischen Faktoren mit geringer Intensität auf den Organismus (Immunological indices as possible criteria in evaluating the effect of chemical factors at low intensity on the organism) (deutsche Übersetzung aus dem Russischen) (German, translation from the Russian). *Азерб Мед Жур Azerb Med Zh* 55: 58–61

Scholpp J, Schubert JK, Miekisch W, Geiger K (2002) Breath markers and soluble lipid peroxidation markers in critically ill patients. *Clin Chem Lab Med* 40: 587–594

Senthilmohan ST, Milligan DB, McEwan MJ, Freeman CG, Wilson PF (2000) Quantitative analysis of tracegases of breath during exercise using the new SIFT-MS technique. *Redox Rep* 5: 151–153

Shamberger RJ (1971) Inhibitory effect of vitamin A on carcinogenesis. *J Natl Cancer Inst* 47: 667–673

Shelby MD, Witt KL (1995) Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ Health Perspect* 25: 302–313

Shelby MD (1990) Results of NTP-sponsored mouse cytogenetic studies on 1,3-butadiene, isoprene, and chloroprene. *Environ Health Perspect* 86: 71–73

Shugaev BB (1969) Concentrations of hydrocarbons in tissues as a measure of toxicity. *Arch Environ Health* 18: 878–882

Sills RC, Hong HL, Melnick RL, Boorman GA, Devereux TR (1999 a) High frequency of codon 61 K-ras A→T transversions in lung and Harderian gland neoplasms of B6C3F1 mice exposed to chloroprene (2-chloro-1,3-butadiene) for 2 years, and comparisons with the structurally related chemicals isoprene and 1,3-butadiene. *Carcinogenesis* 20: 657–662

Sills RC, Boorman GA, Neal JE, Hong HL, Devereux TR (1999 b) Mutations in ras genes in experimental tumours of rodents. In: McGregor DB, Rice JM, Venitt S (1999) The use of short- und medium-term tests for carcinogenesis and data on genetic effects in carcinogenic hazard evaluation, IARC Publ 146, International Agency for Research on Cancer, Lyons, FR, 55–86

Sills RC, Hong HL, Boorman GA, Devereux TR, Melnick RL (2001) Point mutations of K-ras and H-ras genes in forestomach neoplasms from control B6C3F1 mice and following exposure to 1,3-butadiene, isoprene or chloroprene for up to 2 years. *Chem Biol Interact* 135–136: 373–386

Small RD, Golding BT, Watson WP (1997) Species differences in the stereochemistry of the metabolism of isoprene *in vitro*. *Xenobiotica* 27: 1155–1164

Smith D, Španěl P, Davies S (1999) Trace gases in breath of healthy volunteers when fasting and after a protein-calorie meal: a preliminary study. *J Appl Physiol* 87: 1584–1588

Španěl P, Davies S, Smith D (1999) Quantification of breath isoprene using the selected ion flow tube mass spectrometric analytical method. *Rapid Commun Mass Spectrometr* 13: 1733–1738

SRC (Syracuse Research Corporation) (2006) Isoprene, Physprop database, http://esc.syrres.com/fatepointer/search.asp

Statheropoulos M, Sianos E, Agapiou A, Georgiadou A, Pappa A, Tzamtzis N, Giotaki H, Papageorgiou C, Kolostoumbis D (2005) Preliminary investigation of using volatile organic compounds from human expired air, blood and urine for locating entrapped people in earthquakes. *J Chromatogr B Analyt Technol Biomed Life Sci* 822: 112–117

Stein RA, Mead JF (1988) Small hydrocarbons formed by the peroxidation of squalene. *Chem Phys Lipids* 46: 117–120

Stone BG, Besse TJ, Duane WC, Evans CD, DeMaster EG (1993) Effect of regulating cholesterol biosynthesis on breath isoprene excretion in men. *Lipids* 28: 705–708

Sun JD, Dahl AR, Bond JA, Birnbaum LS, Henderson RF (1989) Characterization of hemoglobin adduct formation in mice and rats after administration of [$^{14}$C]butadiene or [$^{14}$C]isoprene. *Toxicol Appl Pharmacol* 100: 86–95

Tareke E, Golding BT, Small RD, Törnqvist M (1998) Haemoglobin adducts from isoprene and isoprene monoepoxides. *Xenobiotica* 28: 663–672

Taucher J, Hansel A, Jordan A, Fall R, Futrell JH, Lindinger W (1997) Detection of isoprene in expired air from human subjects using proton-transfer-reaction mass spectrometry. *Rapid Commun Mass Spectrometr* 11: 1230–1234

Tice RR (1988) The cytogenetic evaluation of *in vivo* genotoxic and cytotoxic activity using rodent somatic cells. *Cell Biol Toxicol* 4: 475–486

Tice RR, Boucher R, Luke CA, Paquette DE, Melnick RL, Shelby MD (1988) Chloroprene and isoprene: cytogenetic studies in mice. *Mutagenesis* 3: 141–146

Turner C, Spanel P, Smith D (2006) A longitudinal study of breath isoprene in healthy volunteers using selected ion flow tube mass spectrometry (SIFT-MS). *Physiol Meas* 27: 13–22

Watson WP, Cottrell L, Zhang D, Golding BT (2001) Metabolism and molecular toxicology of isoprene. *Chem Biol Interact* 135–136: 223–238

Wilkins CK, Clausen PA, Wolkoff P, Larsen ST, Hammer M, Larsen K, Hansen V, Nielsen GD (2001) Formation of strong airway irritants in mixture of isoprene/ozone and isoprene/ozone/nitrogen dioxide. *Environ Health Perspect* 109: 937–941

Wilschut A, ten Berge WF, Robinson PJ, McKone TE (1995) Estimating skin permeation. The validation of five mathematical skin permeation models. *Chemosphere* 30: 1275–1296

Wistuba D, Weigand K, Peter H (1994) Stereoselectivity of *in vitro* isoprene metabolism. *Chem Res Toxicol* 7: 336–343

Wolkoff P, Clausen PA, Wilkins CK, Nielsen GD (2000) Formation of strong airway irritants in terpene/ozone mixtures. *Indoor Air* 10: 82–91

Zadak Z, Hyspler R, Crhova S, Gasparic J, Cizkova M, Balasova V (1999) Isoprene in expired air as a marker of cholesterol synthesis for the statin therapy monitoring. *Atherosclerosis* 144: 206 (Abstract)

Zhang FL, Casey PJJ (1996) Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem* 65: 241–269

completed 05.03.2008

# ISOPRENE
# (Group 2B)

For definition of Groups, see Preamble Evaluation.

**VOL.**: 71 (1999) (p. 1015)

**CAS No.**: 78-79-5
**Chem. Abstr. Name**: 2-Methyl-1,3-butadiene

## 5. Summary of Data Reported and Evaluation

### 5.1 Exposure data

Exposure to isoprene occurs in the production of the monomer and in the production of synthetic rubbers. Isoprene occurs in the environment due to emissions from vegetation and the production of ethylene by naphtha cracking.

### 5.2 Human carcinogenicity data

No data were available to the Working Group.

### 5.3 Animal carcinogenicity data

Isoprene was tested for carcinogenicity in mice and rats by inhalation exposure. In two studies in mice, exposure to isoprene resulted in increased combined incidences of benign and malignant tumours of the lung and liver and of Harderian gland adenomas. In one study, haemangiosarcomas of the heart and spleen and histiocytic sarcomas were also found in male mice, as well as increased incidences of pituitary adenomas and Harderian gland adenomas in female mice. In one adequate study with rats, increased incidences were observed for benign neoplasms in the mammary gland in males and females and in the kidney and testis in males.

### 5.4 Other relevant data

Both rats and mice exhibited saturation kinetics when exposed to concentrations above 300 ppm [840 mg/m$^3$]. The maximal rate of metabolism *in vivo*, which occurs via monoepoxides and diepoxide and subsequent epoxide hydration, is more than three times greater in mice than in rats. In-vitro studies and a physiological toxicokinetic model suggest that the rates of metabolism in humans is lower.

At high inhalation exposures, proliferative lesions in olfactory epithelium and lung were observed. Forestomach epithelial hyperplasia was detected at lower exposure levels in rats and mice. Adverse effects in reproductive organs of male and female mice were detected after high inhalation doses.

Isoprene did not induce mutations in bacteria or sister chromatid exchanges or chromosomal aberrations in animal cells *in vitro*. Isoprene induced sister chromatid exchanges and micronuclei in bone-marrow cells after inhalation exposure of mice.

Isoprene binds covalently to haemoglobin *in vivo*.

### 5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of isoprene were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of isoprene.

**Overall evaluation**

Isoprene is *possibly carcinogenic to humans (Group 2B).*

For definition of the italicized terms, see Preamble Evaluation.

**Previous evaluation**: Vol. 60 (1994)

**Synonym**

- Isopentadiene

---

Last updated: 13 April 1999

# 1,3-Butadiene, 2-methyl-: Human health tier II assessment

**04 July 2014**

## CAS Number: 78-79-5



## Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

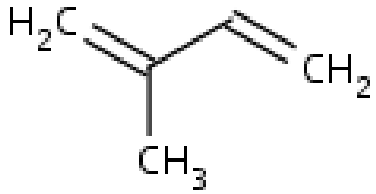
For more detail on this program please visit:www.nicnas.gov.au

**Disclaimer**



Acronyms & Abbreviations

## Chemical Identity

| | |
|---|---|
| Synonyms | 2-methyl-1,3-butadiene<br>isoprene<br>isopentadiene<br>3-methyl-1,3-butadiene<br>beta-methylbivinyl |
| Structural Formula |  |
| Molecular Formula | C5H8 |
| Molecular Weight (g/mol) | 68.12 |
| Appearance and Odour (where available) | Colourless with an aromatic odour. |
| SMILES | C(=C)(C)C=C |

# Import, Manufacture and Use

## Australian

No Australian use, import, or manufacture were reported under previous NICNAS mandatory calls for information.

## International

The following international uses have been identified through European Union Registration, Evaluation and Authorisation of Chemicals (EU REACH) dossiers; the Organisation for Economic Cooperation and Development Screening information data set International Assessment Report (OECD SIAR); Galleria Chemica; Substances and Preparations in the Nordic countries (SPIN) database; the European Commission Cosmetic Ingredients and Substances (CosIng) database; United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; and eChemPortal: OECD High Production Volume chemical program—OECD HPV, the US Environmental Protection Agency's Aggregated Computer Toxicology Resource—ACToR, and the US National Library of Medicine's Hazardous Substances Data Bank—HSDB.

The chemical has reported site-limited use including in the manufacturing of:

- polyisoprene, butyl rubber and styrene-isoprene-styrene (SIS) rubber;
- viscosity improvers for motor oil; and
- speciality chemicals, intermediates and derivatives.

The chemical is found in petrol and other hydrocarbon refinery streams. The chemical is naturally emitted to the atmosphere from various plant and tree species and is also formed endogenously in humans (predominant endogenous hydrocarbon exhaled by humans). The chemical is also present in smoke from cigarettes as well as bushfires and woodfires.

# Restrictions

## Australian

No known restrictions have been identified.

## International

The chemical is listed on the following (Galleria Chemica):

- EU Cosmetics Regulation 1223/2009 Annex I—List of substances prohibited in cosmetic products;
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain isoprene;
- Health Canada List of prohibited and restricted cosmetic ingredients (The Cosmetic Ingredient "Hotlist").

The chemical is also restricted by Annex XVII to REACH Regulation as follows:

Shall not be used in substances and preparations placed on the market for sale to the general public in individual concentration equal to or greater than; either the relevant concentration specified in Annex I to Directive 67/548/EEC, or the relevant concentration specified in Directive 1999/45/EC.

# Existing Work Health and Safety Controls

## Hazard Classification

The chemical is classified as hazardous, with the following risk phrases for human health in the Hazardous Substances Information System (HSIS) (Safe Work Australia):

Carc. Cat. 2; R45  May cause cancer.

Muta. Cat. 3; R68  Possible risk of irreversible effects.

## Exposure Standards

### Australian

No specific exposure standards are available.

### International

The following exposure standards are identified (Galleria Chemica):

An exposure limit, time weighted average (TWA) of 8.5–100 mg/m³ (3–36 ppm) and short-term exposure limit (STEL) of 68–300 mg/m³ (24–50 ppm) in different countries such as Bulgaria, Germany, Latvia, Poland, Russia,  and Switzerland. A WEEL (Workplace Environmental Exposure Limit) of 2 ppm was established by American Industrial Hygiene Association in 2004. The WEEL was 50 ppm prior to the 2004 revision.

# Health Hazard Information

## Toxicokinetics

In mammals, the chemical is metabolised by the microsomal cytochrome P450 (CYP450) dependent monooxygenases in the liver. During metabolism, the double bonds of isoprene are oxidised forming two monoepoxides (EPOX-1 and EPOX-2), which can be further hydrolysed, conjugated with glutathione or further oxidised to the diepoxide. The metabolism of the chemical is saturable.

The metabolism and distribution of isoprene vary among species, with metabolism reported to be faster in mice and Syrian hamsters than in rats and rabbits. These differences in metabolism and reactivity may contribute to the marked species differences in toxicological response to the chemical. In vitro studies and a physiological toxicokinetic model suggest that the rate of metabolism in humans is lower than in rodents (Bogaards et al., 2001; Lofroth et al., 1989; Placke et al., 1996; Csanady and Filser 2001; OECD, 2005; REACH).

## Acute Toxicity

### Oral

The limited data available indicate that the chemical is of low acute toxicity in animals following oral exposure.

In an acute toxicity study conducted in Wistar rats, 15 animals of both sexes were given single doses (250, 500, 1000, 2000, 2150, 2250 and 2500 mg/kg) of isoprene in oil by a stomach tube. The results showed that isoprene doses of >500 mg/kg induced signs of sedation and breathing difficulties one hour after exposure to the chemical. These clinical signs continued to manifest for seven days. The number of deaths occurring, as early as one day after exposure, was strongly correlated with the increasing isoprene doses. There were four, eight, 11 and 15 mortalities at 2000, 2150, 2250 and 2500 mg/kg of isoprene respectively. However, this study was considered limited due to the lack of sufficient experimental details. Based on this data, the median lethal dose (LD50) for isoprene is >2000 mg/kg bw (REACH; OECD, 2005; Government of Canada, 2008).

### Dermal

No reliable data are available.

### Inhalation

Several pre-good laboratory practice (GLP) lethality studies in rats and mice are available. The results of these studies indicate that the chemical is of low acute toxicity in animals following inhalation exposure with reported median lethal concentrations (LC50s) in the range of 139–214 mg/L (REACH; OECD, 2005).

Wistar rats were exposed to six concentrations of isoprene (duration not reported) and the changes in the thymus were evaluated. The results indicated that acute inhalation of 8.40 and 21.41 mg/L of isoprene caused pathological changes in the rats. These include abnormalities in cellularity, mitotic index, absolute and relative weights of the thymus and proliferative activity. However, this study lacked sufficient information for proper evaluation (REACH).

## Corrosion / Irritation

### Respiratory Irritation

Degeneration of the olfactory epithelium was observed in several repeated dose toxicity studies (see **Repeated dose toxicity: inhalation**). Generally, the effects were noted at 1950 mg/m³.

### Skin Irritation

The chemical is reported to have a low potential for skin irritation in animals. The effects were not sufficient to warrant a hazard classification (REACH).

In a skin irritation study, the skin of  two New Zealand White rabbits was painted with 100 % isoprene twice a day for five consecutive days. The results indicated  reversible erythema.

### Eye Irritation

The limited data available indicate that the chemical may be irritating to eyes, there is not sufficient information to warrant hazard classification. According to a non-GLP study, the chemical was reported to cause eye irritation in rats, however no further details were provided.

### Observation in humans

Isoprene vapours are reported to be slightly irritating to the eyes, lungs and skin of humans (OECD, 2005).

In a pre-GLP study, human volunteers experienced slight irritation of the upper respiratory tract following exposure to isoprene at 160 mg/m³; although, there is uncertainty in the reliability of this study due to limited information.

Irritant effects in the upper respiratory tract, mucous membranes and olfactory tract have been reported in workers from a rubber production company. The level and prevalence of the effects were correlated with increasing length of service (IARC, 1994).

These observations, together with the olfactory degeneration observed in the repeated dose animal studies, are considered sufficient to warrant classification for respiratory irritation (see **Recommendation** section).

## Sensitisation

### Skin Sensitisation

No data are available. The chemical does not contain a structural alert for skin sensitisation (OECD Toolbox).

## Repeated Dose Toxicity

### Oral

No data are available.

### Dermal

No data are available.

### Inhalation

Several studies have demonstrated that repeated isoprene exposure by inhalation results in a number of non-cancerous effects, in addition to the increased incidences of multi-organ tumours (see **Carcinogenicity**). In addition to hyperplasia observed at sites at which tumours occurred (lungs and forestomach), effects were generally observed in the haematological system, nasal cavity, liver and testes. Effects in the thymus, spinal cord and spleen were observed in some studies. The available studies also indicated that mice were more susceptible to the damaging effects of inhaled isoprene than rats (Placke et al., 1996; NTP, 1999; OECD, 2005; REACH). These studies and effects are summarised below, with the exception of effects observed in reproductive organs, which are described in the **Reproductive and developmental toxicity** section.

In a two-week repeated dose inhalation study, F344 rats and B6C3F1 mice (20 animals/sex/group/species) were exposed to a series of isoprene concentrations of 0, 1220, 2438, 4876, 9751 or 19503 mg/m³ for six hours a day, five days a week for two weeks. The results showed that in rats, isoprene exposure for two weeks, at any dose, did not cause any pathological changes or incidences of gross or microscopic lesions. This study reported a no observed adverse effect level (NOAEL) of 19503 mg/m³ for rats.

In contrast to the results in rats, the two-week repeated exposure of mice to isoprene caused a reduction in body weight in males from the 19503 mg/m³ group. Dose-related increases in the mean liver weight/body weight ratios; and decreases in relative thymus, and spleen weights were also noted in male mice. Changes in organ weight and haematological profiles were observed in both sexes. Microscopic lesions were present in various organs. These included forestomach lesions in females and epithelial hyperplasia in both sexes (at all doses), thymic atrophy in males dosed at 19503 mg/m³, nasal lesions in animals dosed at 4876 mg/m³ and above (with the severity proportionate to isoprene concentrations) and diffuse liver changes consistent with highly glycogenated hepatocytes in all male dose groups. A NOAEL could not be established for mice in this study.

The findings above were consistent with the data from the subsequent repeated dose inhalation studies.

In a GLP-compliant repeated dose inhalation study, F344 rats and B6C3F1 mice (10/sex/group) were exposed to 0, 195, 613, 1950, 6129, or 19503 mg/m³ of isoprene (whole body inhalation) for six hours a day for five days a week for 13 weeks. The results showed that isoprene exposure did not cause measurable damage in rats. Exposure of male and female mice to 1950 mg/m³ (700 ppm) isoprene or higher induced haematologic abnormalities and forestomach focal epithelial hyperplasia. In male mice, degeneration of the nasal cavity (olfactory epithelium) was noted following exposure to 19503 mg/m³ (7000 ppm) isoprene. Cytoplasmic vacuolisation of hepatocytes due to glycogen accumulation was also observed at the two highest dose levels (OECD, 2005).

In a 26-week inhalation exposure study, B6C3F1 mice and Fischer 344 rats were exposed to 0, 195, 613, 1950, 6129, or 19503 mg/m³ of isoprene vapour by inhalation for six hours a day, five days a week for six months. Animals were evaluated both at 26 weeks and after a further 26-week recovery period. In rats, other than changes to the testes observed at the highest dose (see **Reproductive and developmental toxicity)** there were no observed effects. For mice, in addition to effects observed in the forestomach and nasal cavity (similar to the 13-week study), changes were observed in the lung following the 26-week recovery period. These were manifested as the increased incidence of alveolar epithelial hyperplasia in the 1950 mg/m³ and higher exposure groups. In addition, the incidence of spinal cord degeneration was significantly increased in all exposure groups following the 26-week recovery period and reversible reduction in hindlimb grip strength was observed in the 613 mg/m³ and higher exposure groups. However, these effects on motor function and spinal cord were not observed in chronic carcinogenicity studies in mice and rats.

Non-cancerous effects in the kidney and spleen were observed in a 104-week study in rats (see **Carcinogenicity section**)**.** The incidence of renal tubule hyperplasia was significantly greater in males than in females exposed to 19503 mg/m³ isoprene and the severity of kidney nephropathy was slightly increased in this group. The incidences of splenic fibrosis were also significantly greater in males than in females dosed with isoprene at 1950 mg/m³ and above.

## Genotoxicity

The chemical is classified as hazardous—Category 3 mutagenic substance—with the risk phrase ‘Possible risk of irreversible effects’ (Xn; R68) in HSIS (Safe Work Australia). The available data support this classification.

Isoprene was negative in several in vitro assays, including bacterial mutation, sister chromatid exchanges (SCE) and chromosomal aberration tests in exposed Chinese hamster ovary cells. However, the chemical was genotoxic to mouse bone marrow in vivo (SCE and micronuclei induction) following inhalation exposure. The chemical also produced DNA damage in a comet assay with human peripheral blood mononuclear cells and human leukaemia cells in vitro (Fabiani et al., 2007; Government of Canada, 2008; IARC,1999; NTP, 2011; OECD, 2005).

Neither of the metabolites EPOX-1 and EPOX-2 were mutagenic to *Salmonella typhimurium* TA100 or TA98 when tested up to lethal concentrations (30 mM), whereas another possible minor metabolite, 2-methyl-1,2,3,4-diepoxybutane, was mutagenic in TA100 test (IARC, 1999; NTP, 2011). The metabolite EPOX-1 caused DNA damage in the in vitro comet assay described above (Fabiani et al., 2007).

## Carcinogenicity

The chemical is currently classified as hazardous as a Category 2 carcinogen with the risk phrase ‘May cause cancer’ (T; R45) in HSIS (Safe Work Australia). The available data support this classification.

The International Agency for Research on Cancer (IARC) has classified the chemical as Group 2B (possibly carcinogenic to humans) (IARC, 1999) and the European Commission has classified it as Category 2 (regarded as if they are carcinogenic to man; may cause cancer). The U.S. National Toxicology Program (NTP) has classified isoprene as being reasonably anticipated to be a human carcinogen (NTP, 2011). The above classifications were based on the consistent observations of increased incidences of neoplastic effects of isoprene in multiple organs.

In a carcinogenicity study, male B6C3F1 mice were exposed to isoprene by whole body inhalation at doses of 0, 27.9, 195, 390, 780, 1950, or 6129 mg/m³, four or eight hours a day for five days a week for 20, 40 or 80 weeks. This was followed by recovery/holding periods until the study was terminated at 96 or 104 weeks. Female mice in this study were exposed to 0, 27.9, 195 mg/m³ for 80 weeks and held until week 104. Male mice had significantly increased incidences of tumours in the lung, liver, forestomach and Harderian gland in addition to histiocytic sarcomas and non-significantly increased incidences of heart and

spleen haemangiosarcomas. Increased incidences of Harderian gland and pituitary adenomas (significant) were observed in female mice exposed to the highest concentration (195 mg/m³). For male mice, the low observed effect level (LOEL) appeared to be 1950 mg/m³ for lung tumours and haemangiosarcomas, 780 mg/m³ for malignant forestomach tumours and histiocytic sarcomas, 390 mg/m³ for liver tumours, and 195 mg/m³ for Harderian gland tumours. For female mice, the LOEL appeared to be 195 mg/m³ for all total non-liver, non-lung adenomas and possibly for hemangiosarcomas (Placke et al., 1996; OECD, 2005).

Evidence of carcinogenicity was also apparent in a 26-week repeated dose inhalation study (described in **Repeated dose toxicity: inhalation)** with increased incidences of tumours in several tissues observed at doses of 1950 mg/m³ and higher, including alveolar/bronchiolar adenoma or carcinoma, Harderian gland adenoma, hepatocellular adenoma or carcinoma and forestomach squamous-cell papilloma or carcinoma. There was no clear dose response at higher doses, possibly as a result of saturation of metabolism (IARC, 1995; OECD, 2005).

The NTP report from the two-year inhalation study in F344/N rats provided further evidence of the carcinogenic activity of isoprene (NTP, 1999). In this study, 50 male and female rats were exposed to doses of isoprene at 0, 613, 1950, 19503 mg/m³ for six hours a day, five days a week for 105 weeks. The results from the histopathological evaluations indicated that isoprene caused an increase in incidences of mammary gland fibroadenoma and carcinoma, renal tubule adenoma and hyperplasia, splenic fibrosis, testicular interstitial cell adenoma and a low incidence of rare brain neoplasms (NTP, 1999). Generally, effects were observed at doses of 1950 mg/m³ and higher, although mammary gland fibroadenoma was observed in all exposed females.

Although there is a lack of epidemiological data, isoprene which is a structural analogue of 1,3-butadiene (CAS No. 106-99-0), has been associated in lymphohaematopoietic cancer in exposed workers (NICNAS).

### Reproductive and Developmental Toxicity

Although limited data are available, the information for this chemical and its structural analogue, 1,3 butadiene (CAS No. 106-99-0) show the potential for specific reproductive or developmental toxicity.

In an inhalation study, Swiss CD-1 mice and Sprague Dawley (SD) rats were exposed to 0, 780, 3900, 19503 mg/m³ isoprene for six hours a day, seven days a week during gestational days 617 for mice and days 6–19 for rats. The results showed a marked species difference, such that the isoprene-induced changes were more prominent in mice than in rats. In mice, exposure to all doses of isoprene caused a reduction in foetal body weight. Moreover, a decrease in maternal weight gain and uterine weight and increased incidence in supernumerary ribs were observed in mice which were treated with the highest dose of isoprene (19503 mg/m³). At the two highest doses, the presence of a cleft palate was observed in two of the foetuses. These skeletal changes are considered likely to be secondary to maternal toxicity, although, the effects on foetal bodyweight were observed at all doses and are not considered secondary. Based on the results, a reported NOAEL of 3901 mg/m³ was determined for maternal toxicity and <780 mg/m³ for developmental toxicity in mice (Anderson, 2001; OECD, 2005; REACH).

No specific studies investigating reproductive toxicity were available, although, in a 13-week repeated dose toxicity study (see **Repeated dose toxicity: inhalation**) male mice exposed to isoprene at 19503 mg/m³ exhibited reduced testicular weight, while females showed a significantly longer oestrus cycle. Exposure to isoprene at 1950 and 19503 mg/m³ resulted in atrophy of the seminiferous tubules; and reduced epididymal weights, sperm headcounts and motility (Anderson, 2001; Melnick et al., 1994). Reversible decrease in testis weight and testicular atrophy were also observed in mice following 26 weeks of exposure (see **Repeated dose toxicity: inhalation** for details of the study).

Intraperitoneal injection of 7.34 mmol/kg/bw in 21-day-old B6C3F1 mice led to changes in the ovarian follicles, including reduction of primordial and growing primary to pre-antral follicle counts (IARC, 1994).

Effects observed were reduced foetal bodyweight, testicular atrophy, effects on sperm and damage to ovarian follicles were noted for the structural analogue 1,3-butadiene. There are a number of studies in rats and mice available for this analogue that found reproductive and developmental effects following inhalation exposures to the chemical (NICNAS).

Overall, available data for the chemical, together with effects observed for the analogue 1,3-butadiene, provide sufficient evidence to show that isoprene may have potential reproductive and developmental toxic effects. These are considered sufficient to warrant classification for both reproductive and developmental effects (see **Recommendation** section).

## Risk Characterisation

### Critical Health Effects

The critical health effects for risk characterisation include systemic long-term effects, such as carcinogenicity, mutagenicity, reproductive toxicity and developmental toxicity. A mode of induction for tumours involving direct interaction with genetic material cannot be precluded.

Prolonged exposure to the chemical may also cause respiratory irritation. Although, given the controls required for critical health effects, exposure to irritant concentrations is expected to be unlikely.

### Public Risk Characterisation

A previous mandatory call for information indicated that the chemical was not being introduced into Australia.

Although the chemical may be present in consumer articles introduced into Australia, as a residual monomer, the chemical is expected to be present only in trace amounts (OECD, 2005). Consequently, potential for consumer exposure will be negligible; hence, the public risk from this chemical is not considered to be unreasonable.

### Occupational Risk Characterisation

Although the mandatory call for information indicated that the chemical was not being introduced into Australia, it is expected to be a constituent of a number of UVCB chemicals within the petroleum industry. Therefore, the exposure to isoprene should be controlled. The risks from exposure to isoprene as a constituent of UVCB chemicals will be further considered as part of any IMAP assessment of these chemicals.

The data available support an amendment to the hazard classification in HSIS (refer to **Recommendation** section).

## NICNAS Recommendation

Assessment of the chemical is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

### Regulatory Control

Work Health and Safety

The chemical is recommended for classification and labelling under the current approved criteria and adopted GHS as below. This assessment does not consider classification of physical hazards and environmental hazards.

| Hazard | Approved Criteria (HSIS)[a] | GHS Classification (HCIS)[b] |
|---|---|---|
| Irritation / Corrosivity | Irritating to respiratory system (Xi; R37) | May cause respiratory irritation - Specific target organ tox, single exp Cat. 3 (H335) |
| Genotoxicity | Muta. Cat 3 - Possible risk of irreversible effects (Xn; R68)* | Suspected of causing genetic defects - Cat. 2 (H341) |

| Hazard | Approved Criteria (HSIS)[a] | GHS Classification (HCIS)[b] |
|---|---|---|
| Carcinogenicity | Carc. Cat 2 - May cause cancer (T; R45)* | May cause cancer - Cat. 1B (H350) |
| Reproductive and Developmental Toxicity | Repro. Cat 3 - Possible risk of impaired fertility (Xn; R62) Repro. Cat 3 - Possible risk of harm to the unborn child (Xn; R63) | Suspected of damaging fertility - Cat. 2 (H361f) Suspected of damaging the unborn child - Cat. 2 (H361d) |

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

## Advice for consumers

Products containing the chemical should be used according to label instructions.

## Advice for industry

***Control measures***

Control measures to minimise the risk from inhalation exposure to the chemical should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used. Examples of control measures which may minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker;
- health monitoring for any worker who is at risk of exposure to the chemical if valid techniques are available to monitor the effect on the worker's health;
- air monitoring to ensure control measures in place are working effectively and continue to do so;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

***Obligations under workplace health and safety legislation***

Information in this report should be taken into account to assist with meeting obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((m)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemical are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (m)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of the chemical has not been undertaken as part of this assessment.

## References

Anderson D 2001. Genetic and reproductive toxicity of butadiene and isoprene. Chemico-Biological Interactions 135-136: pp 65-80

Bogaards J, Freidig A, Bladeren P. 2001. Prediction of isoprene diepoxide levels in vivo in mouse, rat and man using enzyme kinetic data in vitro and physiologically-based pharmacokinetic modelling. Chemico-Biological Interactions 138 (3) pp. 247-265

ChemIDPlus, CAS No 78-79-5 http://chem.sis.nlm.nih.gov/chemidplus. Accessed November 2013

Cosmetics Directive (CosIng). Isoprene, (2-Methyl-1,3-butadiene). Accessed November 2013 at http://ec.europa.eu/consumers/cosmetics/cosing

Csanady GA, Filser JG 2001. Toxicokinetics of inhaled and endogenous isoprene in mice, rats and humans. Chemico-Biological Interactions 135-136 pp. 679-685

Fabiani R, Rosignoli P, De Bartolomeo, A, Fuccelli R, Morozzi G 2007. DNA-damaging ability of isoprene and isoprene mono-epoxide (EPOX1) in human cells evaluated with comet assay. Mutation Research 629 pp 7-13

Galleria Chemica. Accessed November 2013. http://jr.chemwatch.net/galleria/

Globally Harmonised System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third edition. Accessed at http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html

Government of Canada 2008. 1,3-Butadiene, 2-methyl- (Isoprene), Screening Assessment for the Challenge. Accessed November 2013 at http://www.chemicalsubstanceschimiques.gc.ca/challenge-defi/batch-lot-2/index-eng.php

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1994. Some industrial chemicals. Isoprene. IARC Monographs on the Evaluation of Carcinogenic Risks to Human. 60: 215-32

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1999. Re-evaluation of organic chemicals, hydrazine, and hydrogen peroxide. Isoprene. IARC Monographs on the Evaluation of Carcinogenic Risks to Human 71 (3): 1015-25

Lofroth G, Burton, R, Forehand L, Hammond SK, Sella RL, Zweldinger RB, Lewtas J 1989. Characterisation of environmental tobacco smoke. Environmental Science and Technology 23 (5), pp 610-614

Melnick R, Sills R, Roycroft J, Ragan H, Miller R 1994. Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. Cancer Research 54: pp 5333-5339

National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Human health Tier II assessment for 1,3-butadiene. Australian Government Department of Health. Accessed December 2013 at http://www.nicnas.gov.au

NTP 2011. NTP Report on the Carcinogens, Twelfth Edition. Isoprene CAS 78-79-5. Accessed November 2013 at http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/Isoprene

NTP. 1999. NTP Report on the Toxicology and Carcinogenesis Studies of Isoprene (CAS No.78-79-5) in F344/N Rats (Inhalation Studies). NTP Technical Report Series 486. Research Triangle Park, NC: National Toxicology Program pp. 178 (http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr486.pdf)

OECD (2005). SIDS Initial Assessment Profile (SIAP) on Isoprene. Accessed at http://webnet.oecd.org/HPV/UI/handler.axd?id=2d19a70d-10ec-4ff9-9b9a-0978d0db8798

OECD QSAR Toolbox version 3.1. http://www.oecd.org/chemicalsafety/assessmentofchemicals/theoecdqsartoolbox.htm

Placke ME, Griffis L, Bird M, Bus J, Persing RL, Cox LA Jr 1996. Chronic inhalation oncogenecity study of isoprene in B6C3F1 mice. Toxicology 110 pp. 253-262.

REACH Dossier. Isoprene. Accessed November 2013 at http://apps.echa.europa.eu/registered/data/dossiers/

Safe Work Australia (SWA). Hazardous Substances Information System (HSIS). Accessed November 2013 at http://hsis.safeworkaustralia.gov.au/HazardousSubstance

Safe Work Australia (SWA). Hazardous Substances Information System (HSIS). Accessed November 2013 at http://hsis.safeworkaustralia.gov.au/HazardousSubstance

Last update 04 July 2014

Share this page

FOREWORD INTRODUCTION

# ISOPRENE

CAS N°: 78-79-5

# SIDS Initial Assessment Report

# For

# SIAM 20

Paris, France, 19-22 April 2005

| | |
|---|---|
| **1. Chemical Name** | Isoprene |
| **2. CAS Number** | 78-79-5 |
| **3. Sponsor Country** | United States<br>National SIDS Contact Point in Sponsor Country:<br>Oscar Hernandez, Director<br>U.S. Environmental Protection Agency<br>Risk Assessment Division (7403 M)<br>1200 Pennsylvania Avenue, NW<br>Washington DC 20460<br>Phone: (202) 564-7461 |
| **4. Shared Partnership** | Olefins Panel, American Chemistry Council |
| **5. Roles/Responsibilities of the Partners** | |
| • Name of industry sponsors /consortium | Olefins Panel, American Chemistry Council / Elizabeth Moran<br>ExxonMobil Biomedical Sciences, Inc. / Dan Caldwell |
| • Process used | Documents drafted by ExxonMobil Biomedical Sciences, Inc. P.O. Box 971, 1545 Route 22 East, Annandale, NJ 08801-0971, USA. Reviewed by Olefins Panel, American Chemistry Council industry toxicologists and by United States Competent Authority. |
| **6. Sponsorship History** | |
| • How was the chemical or category brought into the OECD HPV Chemicals Programme? | Industry sponsored the assessment of isoprene under the ICCA Initiative with agreement by the United States (US) to act as sponsor country. |
| **7. Review Process Prior to the SIAM** | Industry prepared documents intended for consideration at SIAM 20 that were peer-reviewed within industry. The industry draft dossier, SIAR, and SIAP were submitted to the US, Environmental Protection Agency (EPA) for review prior to their posting on the CDG. Comments from Competent Authorities on the submitted documents received through the CDG will be reviewed by industry and the EPA, and appropriate changes applied. |

| | |
|---|---|
| **8. Quality Check Process** | **Industry Consortium:**<br>Critical biological studies discussed in the SIAR were reviewed for quality by industry and assigned a reliability code, based on the review process guidance of Klimisch *et al.* (1997). Robust summaries of critical data were added to a SIDS dossier for isoprene and flagged as "critical", the summary formats for selected endpoints were largely based on descriptions in the OECD Form and Guidance for preparing and submitting the SIDS DOSSIER (INCLUDING ROBUST STUDY SUMMARIES), which is from the Manual for Investigation of HPV Chemicals.<br><br>**US Government:**<br>US EPA peer-reviewed the SIDS documents and audited selected key studies to check the robust study summaries. |
| **9. Date of Submission** | 21 January 2005 |
| **10. Date of Last Update** | 29 July 2005 |
| **11. Comments** | None |

## SIDS INITIAL ASSESSMENT PROFILE

| | |
|---|---|
| **CAS No.** | 78-79-5 |
| **Chemical Name** | 1,3-Butadiene, 2-Methyl- (Isoprene) |
| **Structural Formula** | $C_5H_8$ ($CH_2$=C($CH_3$)-CH=$CH_2$) |

## SUMMARY CONCLUSIONS OF THE SIAR

**Human Health**

1,3-Butadiene, 2-methyl- (isoprene) is formed endogenously at the rate of 1.9 umol/kg per hour in both rats and mice and at the rate of 0.15 umol/kg per hour in humans. Isoprene is metabolized by microsomal cytochrome P-450 dependent monooxygenases to two monoepoxide metabolites, i.e., 3,4-epoxy-3-methyl-1-butene (EPOX-1) and 3,4-epoxy-2-methyl-1-butene (EPOX-2). These metabolites may then be hydrolyzed, conjugated with glutathione, or further oxidized to the isoprene diepoxide, i.e., 2-methyl-1,2:3,4-diepoxybutane. The intrinsic rates of formation of monoepoxides in human, rat and mouse liver microsomes are roughly similar, when epoxide hydrolase is inhibited, whereas the amount of monoepoxides at the end of incubation can be two to 15 times higher in mouse liver microsomes than in rat and human liver microsomes, respectively. A physiological toxicokinetic model has been developed for inhaled isoprene in mice, rats and humans, taking into account published or assumed kinetic parameters. On the basis of this model, at human exposure conditions (up to 50 ppm [140 mg/$m^3$]), rates of metabolism are about 14 times faster in mice and about eight times faster in rats than in humans. As the epoxide metabolites are likely responsible for the toxic effects of isoprene, this may explain why the mouse is more susceptible to isoprene toxicity than the rat.

Isoprene has a low potential for acute toxicity. In rats and mice, the oral $LD_{50}$ of isoprene is in the range of 2,043 to 2,210 mg/kg. The 4-hour rat $LC_{50}$ is 64,620 ppm (180,037 mg/$m^3$) and the 2-hour mouse $LC_{50}$ is 56,363 ppm (157,033 mg/$m^3$). In humans, isoprene vapors are irritating to the eyes, nose and throat. Liquid isoprene is irritating to the eyes and skin. Data from a 13-week repeated dose study conducted in mice and rats found degeneration of the olfactory epithelium in male mice only at the highest concentration, i.e., 7,000 ppm (19,503 mg/$m^3$), but not at lower concentrations. IARC reports that in isoprene rubber production workers, subtrophic and atrophic processes in the upper respiratory tract, catarrhal inflammation, and degeneration of the olfactory tract were observed. Prevalence and degree were correlated with increasing length of service.

Repeated dose studies demonstrate clear species differences between rats and mice in susceptibility to isoprene. In a 2-week repeated dose inhalation study, the NOAEL for rats was 7,000 ppm (19,503 mg/$m^3$), the highest dose tested. However, in this same study, exposure of mice to isoprene produced changes in hematological parameters(decreased hematocrit, hemoglobin, erythrocytes), body and organ weights (increased liver weights, decreased thymus, spleen and testes weights) and also produced microscopic lesions in certain tissues (testes, thymus, liver, nasal cavity, forestomach) at levels as low as 438 ppm (1,220 mg/$m^3$). Thus, 438 ppm was the LOAEL for mice. Similarly, in the 13-week repeated dose inhalation study, the NOAEL for rats was 7,000 ppm (19,503 mg/$m^3$). In mice, however, hematological effects indicative of a nonresponsive macrocytic anemia and histopathological changes (forestomach, olfactory epithelium, liver) were observed at exposures of 700 ppm (1,950 mg/$m^3$) and higher. The NOAEL for mice in the 13- week repeated dose study was 220 ppm (613 mg/$m^3$). Isoprene was tested for mutagenicity in a series of *in vivo* and *in vitro* studies. Isoprene was not genotoxic in any of the *in vitro* assays conducted. However, when exposed by inhalation, isoprene was clearly genotoxic to mouse bone marrow *in vivo*.

Two-year inhalation carcinogenicity studies were conducted with isoprene in B6C3F1 mice and F344 rats. There is clear evidence of carcinogenicity of isoprene in mice. Isoprene produced exposure-related increases in the incidence of malignant neoplasms in the liver, lung, Harderian gland and forestomach of mice, as well as increases in the number of hemangiosarcomas and histiocytic sarcomas. In rats, there were no significant increases in the incidence of malignant tumors. Isoprene exposures in rats were associated with increases in the rates of benign tumors in the testes and kidney (male) and mammary gland (male and female). Although single incidences of several rare brain neoplasms were observed in female rats, the fact that they were of several distinct cell types makes it difficult to

determine if they are truly exposure related. Based on the carcinogenicity studies conducted in mice and rats, the NTP listed isoprene as reasonably anticipated to be a human carcinogen and IARC has classified it as 2B; possibly carcinogenic to humans.

Isoprene did not produce any maternal or developmental toxicity in rats following exposure to concentrations as high as 7000 ppm. However, both maternal and developmental toxicity were evident in mice. In mice, both maternal weight gain and uterine weight were significantly reduced at the highest dose (i.e., 7000 ppm). Significant reductions in fetal bodyweights were observed at the 280 ppm dose level for female fetuses and at the 1400 ppm level for male fetuses. Thus, in this study, 1400 ppm was the NOAEL for maternal toxicity. A NOAEL for developmental toxicity could not be determined as effects were observed at the lowest exposure concentration tested, i.e., 280 ppm.

Isoprene did not produce any significant effects on reproductive endpoints in rats. However, significant effects on reproductive endpoints were observed in male mice exposed to isoprene at concentrations of 700 ppm (1,950 mg/$m^3$) and higher. These effects included testicular atrophy as well as decreases in epididymal weight, sperm head count, sperm concentration, and sperm motility. In female mice exposed to 7,000 ppm (19,503 mg/$m^3$ ), the average estrous cycle length was significantly longer than that of the control group. Thus, in this study, 70 ppm (195 mg/$m^3$) is the NOAEL for reproductive effects in male mice and 700 ppm (1,950 mg/$m^3$) is the NOAEL for female mice.

**Environment**

Isoprene is a liquid at 25° C with a reported melting point of –145.9° C, a boiling point of 34.0° C, and vapour pressure of 733.3 hPa (25° C). Isoprene has a water solubility of 642 mg/l (25° C), a log $K_{ow}$ of 2.42, and a density of 0.681 g/$cm^3$ (25° C).

In the air, isoprene has the potential to rapidly degrade through indirect photolytic processes mediated primarily by hydroxyl radicals with a calculated degradation half-life of 1.2 hours depending on hydroxyl radical concentration. Aqueous photolysis and hydrolysis will not contribute to the transformation of isoprene in aquatic environments because it is either poorly or not susceptible to these reactions.

Results of Mackay Level I distribution modeling at steady state show that isoprene will partition primarily to the air compartment (99.9%), with a negligible amount partitioning to water (0.06%) and soil (0.02%). Level III modeling predicted greatest distribution (99.96%) to the primary compartment of release; when equal releases were assumed, the predicted distribution was: water (88%), soil (9.0%), air (3.1%), and sediment (0.21%).

Isoprene biodegraded to 61 % after 28 days in an OECD 301F study, but was not readily biodegradable because the replicate data exceeded the allowable range (53 to 75%). In an OECD 301D study, isoprene biodegraded to an extent of 2 and 58% in duplicate samples after 28 days, and showed no inhibitory effect in a supplementary study. The supplementary study resulted in 64% biodegradation on day 7, using the acclimated inoculum from the initial study. These data show that isoprene can exhibit high extents of biodegradation once acclimation has occurred. Bioaccumulation of isoprene is unlikely based on a low potential to bioconcentrate. The measured BCF is reported as 5 to approximately 20. The calculated BCF is 15.

Acute aquatic toxicity values for a fish and invertebrate are 7.4 (96hr-$LC_{50}$) and 5.8 (48hr-$EC_{50}$) mg/L, respectively. For algae, the 72- and 96-hr $EC_{50}$ is 15 mg/L for biomass and >35 mg/L for growth rate. The algae 72- and 96-hr NOEC is 1.7 and 6.0 mg/L for biomass and growth rate, respectively.

**Exposure**

Isoprene is a petrochemical that is used as a chemical intermediate to manufacture primarily polymers, which occurs in closed production systems. Greater than 95% of high-purity isoprene is used as a monomer to manufacture elastomers such as polyisoprene, styrenic thermoplastic elastomer block copolymers (styrene-isoprene-styrene [SIS]), and butyl rubber. The remaining amount of isoprene is used to manufacture specialty chemicals, intermediates and derivatives which are then used in the production of vitamins, pharmaceuticals, flavorings and perfumes, and epoxy hardeners. The European Union has evaluated isoprene in the framework of Food Contact Material (CS/PM/3351/21640).

Total world isoprene consumption was reported as over 700,000 metric tons in 2004. Most isoprene production is consumed in the country of origin. Isoprene world consumption in 2000 was 579,000 metric tons, of which approximately 96% was consumed in the country of manufacture. In the United States, isoprene production in 1995

was approximately 619 million lb (281,000 metric tons).

Potential occupational exposure to isoprene through inhalation and dermal contact could occur at workplaces where isoprene or synthetic rubber is produced or used. A WEEL (Workplace Environmental Exposure Limit) of 2 ppm was established by AIHA in 2004. The WEEL was 50 ppm prior to the 2004 revision. Isoprene concentration in 426 workplace air samples (4-hr or greater) taken at 3 major isoprene or isoprene polymer producers in the United States from 1993 to 1998 showed that 81% were below 0.5 ppm, 91% were below 1 ppm, and 99% were below 10 ppm.

There are no direct sales to consumers. However, isoprene is used in production of polymers used in paint resins, tyres, footwear, moduled goods, adhesives, motor oil viscosity improvers. Isoprene monomer residual concentration was not detectable in isoprene-derived polymer samples at an analytical sensitivity of 0.1 ppm in work conducted prior to June 1998. Subsequent work in latter 1998, with an increased analytical sensitivity of 0.02 ppm, that evaluated polyisoprene samples demonstrated that 17 out of 19 samples had no detectable isoprene monomer residual, while 2 samples contained between 0.04 and 0.02 ppm. Consequently, potential for consumer exposure will be negligible.

The greatest potential for exposure to isoprene in the environment is in the air compartment because of its high vapor pressure. Partitioning to air from aquatic and terrestrial compartments would occur rapidly due to isoprene physico-chemical characteristics. As such, isoprene has an overall low potential for exposure in environmental compartments other than air. However, its persistence in air is short lived as a result of degradation processes, which suggests that exposure to isoprene will be limited in the environment.

## RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

The chemical is currently of low priority for further work. The chemical possesses properties indicating a hazard for human health (irritation, genotoxic, reproductive and developmental toxicity, carcinogenic) and the environment (fish, invertebrates, algae). Based on data presented by the Sponsor country, relating to production in one country which accounts for approximately 40% of global production and relating to the use pattern in one country, under normal manufacturing, formulation, industrial and consumer use of polymerized isoprene containing products, this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

# SIDS Initial Assessment Report

## 1 IDENTITY

### 1.1 Identification of the Substance

| | |
|---|---|
| CAS Number | 78-79-5 |
| IUPAC Name | 1,3-Butadiene, 2-Methyl- |
| Molecular Formula | $C_5H_8$ |
| Structural Formula | CH3<br>\|<br>CH2=C-CH=CH2 |
| Molecular Weight | 68.12 |
| Synonyms | Isoprene; 2-Methyl-1,3-Butadiene; 2-Methylbutadiene; 3-Methyl-1,3-Butadiene, Methylbivinyl; Beta-Methylbivinyl; Hemiterpene |

### 1.2 Purity/Impurities/Additives

Isoprene purity is 99+% w/w. Impurities in isoprene can include isoprene dimer (dipentene) at less than or equal to 0.5 % w/w. Isoprene can contain 150 to 250 ppm p-tert butyl catechol added as a stabilizer.

### 1.3 Physico-Chemical Properties

**Table 1. Summary of Physico-Chemical Properties for Isoprene**

| Property | Value | Reference/Comment |
|---|---|---|
| Physical state (25° C) | Liquid | None |
| Molecular Weight | 68.12 (c) | None |
| Melting point (°C) | -145.9 (m) | O'Neil et al. (2001) |
| Boiling point (°C) | 34.0 (m) | O'Neil et al. (2001) |
| Relative density ($g/cm^3$ at 20° C) | 0.681 (m) | Budavari S (1996) |
| Vapor pressure (hPa at 25° C) | 733.3 (m) | Zwolinski and Wilhoit (1971) |
| Water solubility (mg/l at 25° C ) | 642 (m) | McAuliffe (1966) |
| Partition coefficient n-octanol/water (log $K_{ow}$) | 2.42 (m) | CITI (1992) |
| Henry's Law constant (HLC) ($Pa*m^3$/mole at 25° C) | 7,781 (c) | HLC was calculated using a water solubility of 642 mg/L, vapor pressure of 733.3 hPa, and molecular weight of 68.12. |
| Partition coefficient organic carbon/water (log $K_{oc}$) | 1.83 (c) | EPIWIN (1999) |

(m) measured (c) calculated

# 2 GENERAL INFORMATION ON EXPOSURE

Exposure to isoprene may occur at workplaces where it is manufactured. Based on physical properties, the primary workplace exposure would be by inhalation. No consumer exposure is foreseen because there are no direct sales to consumers.

## 2.1 Production Volumes and Use Pattern

Isoprene is used as a chemical intermediate to manufacture primarily polymers, which occurs in closed production systems. Greater than 95% of high-purity isoprene is used as a monomer to manufacture elastomers such as polyisoprene, styrenic thermoplastic elastomer block copolymers (styrene-isoprene-styrene [SIS]), and butyl rubber. The remaining amount of isoprene is used to manufacture specialty chemicals, intermediates and derivatives, which are then used in the production of vitamins, pharmaceuticals, flavorings and perfumes, and epoxy hardeners. Coblockpolymers (SIS polymers) are used in the production of paint resins, tires, footwear, moduled goods, adhesives, and motor oil viscosity improvers. Also of commercial importance is the chemical conversion of relatively small amounts of isoprene to terpens which are used extensively in flavours and fragrances (Taalman, 1996).

Isoprene world consumption in 2000 was 579,000 metric tons, of which approximately 96% was consumed in the country of manufacture (SRI International, 2000). In the United States, isoprene production in 1995 was approximately 619 million lb (281,000 Mg [metric tons) (USITC, 1995).

Isoprene is obtained by extractive distillation from an isoprene concentrate stream produced by the ethylene production process. In the pyrolysis furnaces of the ethylene production process, paraffinic feedstocks such as ethane, propane, naphthas or gas oils, are subjected to high temperatures in the presence of steam. These conditions result in the partial conversion or cracking of the hydrocarbon feedstock components and formation of unsaturated hydrocarbons. Ethylene and propylene are the primary products, but other olefins, diolefins, aromatics and cyclics are also produced, including a relatively small amount of isoprene. The ethylene process compresses and separates the pyrolysis furnace effluent into product streams. Isoprene produced in the cracking furnace is contained in one of these product streams, the pyrolysis gasoline.

Pyrolysis gasoline is a complex hydrocarbon mixture, consisting predominately of carbon number five (C5+) and higher hydrocarbon components. Distillation of pyrolysis gasoline produces a pyrolysis C5 stream. This stream is also a complex mixture, and consists predominately of the carbon number 5 olefins and diolefins that were produced in the ethylene process cracking furnaces. The stream also includes n-pentane and iso-pentane, which may result largely due to the unconverted pentanes in the ethylene process feedstock. Further processing of the C5 stream results in an isoprene concentrate stream that is separated from the Pyrolysis C5 by a series of distillation and “heat soak” operations. The “heat soak” is used to convert cyclopentadiene to its dimer (dicyclopentadiene) in order to facilitate isolation of the isoprene concentrate. Isoprene concentrate thus produced from the pyrolysis C5 stream has a typical isoprene content of 40%. This concentrate is then processed in an extractive distillation unit that uses a solvent such as acetonitrile to facilitate isolation of the contained isoprene as a 99% purity product.

Isolation of isoprene from the ethylene process co product streams as described above is the primary source of isoprene. “Only this method of production is practiced in the United States and Western Europe. On-purpose synthetic routes to isoprene are also used commercially, including dehydrogenation of isoamylene and isopentane (capacity in Russia) and reaction of isobutylene with formaldehyde (Russia and Japan)” (Kaelin *et al.*, 2005).

## 2.2 Environmental Exposure and Fate

Isoprene will partition largely into the atmosphere because it has a relatively high vapor pressure at ambient temperatures. Results from an equilibrium distribution model support that isoprene will partition predominantly to the air compartment. Once in the air, wet deposition of isoprene from the air is not likely to play a significant role in its atmospheric fate due to its short half-life in air. In the air isoprene has the potential to rapidly degrade through an indirect photolytic process mediated by hydroxyl radicals (•OH). In comparison, direct photolysis as well as hydrolysis are not expected to contribute to the removal of isoprene from the environment because it is not subject to these degradative processes. Isoprene has the potential to biodegrade to a significant extent based on results of ready biodegradation testing. However, microbial metabolism may not greatly contribute to its removal from the environment because of its potential to rapidly volatilize from aquatic and terrestrial media. Bioaccumulation of isoprene is unlikely based on a low potential to bioconcentrate.

### 2.2.1 Sources of Environmental Exposure

Higher plants emit volatile hydrocarbons including isoprene and monoterpenes into the atmosphere. The world wide emission rate of these natural hydrocarbons has been estimated to range from 1.8 to 8.3 E11 kg/yr, which exceeds that of non methane hydrocarbons originating from human sources (Brookhaven National Laboratory, http://www.face.bnl.gov/Modelling/isoprene.htm). Typically, 1-2% of $CO_2$ fixed in photosynthesis is emitted as isoprene, and globally it is estimated that 2.72 to 3.63 E11 kg of isoprene is emitted by forests each year (Fall, http://www.colorado.edu/Chemistry/directory.dir/faculty.dir/biochem.dir/fall.dir/fallres.html).

Presumably the expected exposure from industrial or anthropogenic sources of isoprene throughout the industrial life-cycle would be less than that from natural sources.

Daily isoprene emission rates positively correlate with leaf temperature, which suggests that there is a correlation with time of day. Emissions of isoprene in large forested regions may be significant to the extent that oxidative/reductive balances are influenced. Plant-emitted isoprene contributes to rural ozone concentrations in summer (Guenther *et al.*, 1991; Mendis, 1994; Monson *et al.*,1994). Isoprene polymers also occur naturally. The natural rubber caoutchouc is *cis* -1,4-polyisoprene, and *trans* -1,4-polyisoprene is present in the natural rubbers balata and gutta-percha. (Columbia Encyclopedia, 2005; http://www.encyclopedia.com/html/i1/isoprene.asp).

Song *et al.* reported on sources of isoprene in Harris County, TX, USA (Table 2) from the Texas Air Quality Study (TexAQS, www.utexas.edu/research/ceer/texaqs) conducted in August and September 2000.

**Table 2. Anthropogenic sources of isoprene in Harris County, TX, USA**

| **Point Source Emissions** | **Tons/Day** | **Percent (%)** |
|---|---|---|
| Chemical Manufacturing | 0.21714 | 54.46 |
| Secondary Metal Production | 0.00002 | 0.005 |
| Mineral Products | 0.00048 | 0.12 |
| Petroleum Industry | 0.02594 | 6.51 |
| Fabricated Metal Products | 0.00283 | 0.71 |
| Printing and Publishing | 0.00056 | 0.14 |
| Surface Coating Operations | 0.00053 | 0.13 |
| Petroleum Product Storage at Refineries | 0.08927 | 22.39 |
| Petroleum Liquids Storage(non-Refinery) | 0.05041 | 12.64 |
| Organic Chemical Storage | 0.00163 | 0.41 |
| Organic Chemical Transportation | 0.00771 | 1.93 |
| Organic Solvent Evaporation | 0.00218 | 0.55 |
| | | |
| **Emissions** | **Tons/Day** | **Percent (%)** |
| Point sources | 0.6772 | 70.26 |
| Mobile sources | 0.2303 | 23.89 |
| Area sources | 0.0013 | 0.13 |
| Off-road sources | 0.0551 | 5.72 |
| **Total** | **0.9639 (= 874 kg/day)** | **100** |

### 2.2.2 Photodegradation

In air, a chemical can react with photosensitized oxygen in the form of $^{\bullet}$OH. This reaction is characterized as indirect photodegradation, and can result in a parent chemical's complete degradation. Isoprene rapidly reacts with $^{\bullet}$OH in air.

Potential $^{\bullet}$OH reaction rates with isoprene and the atmospheric chemical half-life can be calculated based on an average $^{\bullet}$OH radical concentrations. The atmospheric oxidation potential model (Meylan and Howard, 1993) calculates a rate constant of 105.14 x $10^{-12}$ $cm^3mol^{-1}s^{-1}$ for isoprene and an average isoprene atmospheric half-life ($t_{½}$) of 1.2 hours or 0.1 days based on a 12-hour light period in a day (the 12-hour day half-life value normalizes degradation to a standard day light period during which hydroxyl radicals needed for degradation are generated). This value is calculated using an average global $^{\bullet}$OH concentration of $1.5x10^6$ $^{\bullet}OH/cm^3$ (EPIWIN, 1999). These data indicate that indirect photodegradation can significantly contribute to the degradation of isoprene in the environment.

In comparison, direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then the resultant excited state of the chemical may undergo a transformation. A prerequisite for direct photodegradation is the ability of one or more bonds within a chemical to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break

chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer.

An approach to assessing the potential for a substance to undergo photochemical degradation is to assume that degradation will occur in proportion to the amount of light absorbed by the chemical at wavelengths >290 nm (Zepp and Cline, 1977). Isoprene does not absorb light within a range of 290 to 750 nm. Therefore, photolysis does not contribute to the degradation of isoprene in the aquatic environment.

### 2.2.3 Stability in Water

Results from equilibrium distribution modeling (Mackay Level I and Level III; see next Section 2.2.4) show that isoprene has the potential to partition to water at significant concentrations only when emitted to this compartment. However, the levels of isoprene that may occur in aquatic environments are unlikely to degrade by hydrolysis because it lacks a functional group that is hydrolytically reactive (Gould, 1959; Harris, 1982). Therefore, this degradative process will not contribute to the removal of isoprene from the environment.

### 2.2.4 Transport between Environmental Compartments

Fugacity-based multimedia modelling provides basic information on the relative distribution of a chemical between selected environmental compartments (i.e., air, soil, water, sediment, suspended sediment, and biota). Widely used fugacity models are the EQC (Equilibrium Criterion) Level I and Level III models (Mackay, 1996). These models require the input of basic physicochemical parameters (i.e., molecular weight, melting point, vapor pressure, water solubility, log $K_{ow}$). The Level III model also requires the input of emission rate and half-life data.

Results of the Mackay Level I environmental distribution model (Table 2) show that isoprene has the potential to partition primarily to air, with a negligible amount partitioning to water and soil. These results can be explained by isoprene's high vapour pressure, 733.3 hPa at 25°C (EPIWIN, 1999). Whereas, Level III modeling (Table 3) indicates that isoprene partitions mostly to the water compartment rather than air compartment when an equal emission rate (1000 kg/hr) to each compartment is assumed. The shorter half-life in the air compartment relative to other compartments contributes to this result. When releases occur only to each of the air and water compartments, independent of one another, isoprene is indicated in the modeling to partition primarily to those compartments, respectively. However, Level III modeling is unlikely to be representative of the ultimate disposition of isoprene because a default emission rate (1000 kg/hr) was used in the model and is not representative of actual chemical discharge.

**Table 3. Environmental distribution as calculated by the Mackay (1998) Level I fugacity model**

| Environmental Compartment | Percent Distribution* |
|---|---|
| Air | 99.92 |
| Water | 0.06 |
| Soil | 0.02 |
| Sediment | 0.00 |
| Suspended Sediment | 0.00 |
| Biota | 0.00 |

*Distribution is based on the following model input parameters (from Table 1):

| | |
|---|---|
| Molecular Weight | 68.12 |
| Temperature | 25° C |
| Log $P_{ow}$ | 2.42 (measured value) |
| Water Solubility | 642 g/$m^3$ (measured value) |
| Vapor Pressure | 733.3 hPa (measured value) |
| Melting Point | -145.9° C (measured value) |

**Table 4. Environmental distribution as calculated by the Mackay (1998) Level III fugacity model**

| Environmental Compartment | Percent Distribution* (equal emission rate to each compartment, 1000 kg/hr**) | Percent Distribution* (releases only to the air compartment, 1000 kg/hr) | Percent Distribution* (releases only to the water compartment, 1000 kg/hr) |
|---|---|---|---|
| Air | 3.11 | 99.96 | 0.42 |
| Water | 87.72 | 0.02 | 99.34 |
| Soil | 8.96 | 0.02 | 0.00 |
| Sediment | 0.21 | 0.00 | 0.24 |

*Distribution is based on the following model input parameters (from Table 1):

| | |
|---|---|
| Molecular Weight | 68.12 |
| Temperature | 25° C |
| Log $P_{ow}$ | 2.42 (measured value) |
| Water Solubility | 642 g/$m^3$ (measured value) |
| Vapor Pressure | 733.3 hPa (measured value) |
| Melting Point | -145.9° C (measured value) |
| Degradation Half-lives (hr): | |
| Air | 1.2 |
| Water | 120 |
| Soil | 420 |
| Sediment | 420 |

**Emission rate is 1000 kg/hr into each of air, water, and soil compartments.

### 2.2.5 Biodegradation

Isoprene was shown to biodegrade to an extent of 60.9% after 28 days based on a study that used the OECD 301F, manometric respirometry, test guideline (ExxonMobil Biomedical Sciences, Inc., 2004), but was not readily biodegradable because the replicate data exceeded the allowable range

(53 to 75%). In an OECD 301D study, isoprene biodegraded to an extent of 2 and 58% in duplicate samples after 28 days, and showed no inhibitory effect in a supplementary study. The supplementary study resulted in 64% biodegradation on day 7, using the acclimated inoculum from the initial study.

Based on scientific judgement that considered estimated aerobic biodegradation half-life values, the biodegradation half-life of isoprene in soil is estimated to range from 7 to 28 days (Howard *et al.*, 1991).

### 2.2.6 Bioaccumulation

Measured log bioconcentration factor (BCF) values are reported as 0.7 to 1.1 (BCF = 5.0 to 14) and 0.7 to 1.3 (BCF = 5.6 to 20) at isoprene exposure concentrations of 50 and 5 mg/L, respectively (CITI, 1992). A log BCF of 1.16 (BCF = 14.6) is calculated (EPIWIN, 1999) with respect to the log $P_{ow}$ = 2.42. Isoprene in the aquatic environment is expected to demonstrate a low potential for bioaccumulation.

### 2.2.7 Other Information on Environmental Fate

Isoprene has the potential to rapidly volatilize from surface waters, based on a Henry's Law constant (HLC) representing volatility of 7,781 Pa-$m^3$/mole. The HLC was calculated using a water solubility of 642 mg/L, a vapor pressure of 733.3 hPa, and a molecular weight of 68.12. The volatilisation half-life of isoprene from a model river and lake is estimated to be approximately 0.8 hours and 3.3 days, respectively (EPIWIN, 1999). Isoprene is not expected to sorb significantly to organic matter in soil, sediment, and wastewater solids based on a log $K_{oc}$ of 1.83 (EPIWIN, 1999).

The photochemical ozone creation potential (POCP) index for a chemical provides a relative measure of its reactivity or ozone forming potential. The POCP index can also provide a means of ranking volatile organic compounds (VOCs) by their ability to form ozone in the troposphere. Reported POCP indices for isoprene in northwestern Europe range from 109.2 to 117.8 (Derwent *et al.*, 1996; Derwent *et al.*, 1998), in comparison with a POCP index of 100 for ethylene, the reference substance.

Isoprene can react easily with hydroxyl radicals. The atmospheric life-time is less than 1 day. Isoprene does not have Cl- or Br-atoms. Therefore, reactive Cl- or Br-substances, which can have an adverse impact on stratospheric ozone concentration, are not formed following photochemical degradation and the ozone depletion potential of this substance is considered negligible. When considered with isoprene's relatively short atmospheric half-life, its contribution to global warming can be considered minor.

## 2.3 Human Exposure

### 2.3.1 Occupational Exposure

Isoprene is a petrochemical that is used as a chemical intermediate in contained systems. Potential occupational exposure to isoprene through inhalation and dermal contact could occur at workplaces where isoprene or synthetic rubber is produced or used. The National Institute for Occupational Safety and Health (NIOSH) estimated that 3,654 workers (578 of these are female) were potentially

exposed to isoprene in the US (from a National Occupational Exposure Survey 1981 to 1983) (NIOSH, 1989).

The following are reported occupational exposure limits:

- MAC (time weighted average) = 100 mg/$m^3$, Poland (RTECS)
- MAC (short-term exposure limit) = 300 mg/$m^3$, Poland (RTECS)
- Short term exposure limit = 40 mg/$m^3$, Russia (International Labour Office, 1983)
- Workplace Environmental Exposure Level (WEEL): 8-hr Time-weighted Average (TWA) = 2 ppm, United States (AIHA, 2005)

Catarrhal inflammation, subtrophic, and atrophic processes in the upper respiratory tract, as well as deterioration of olfaction were noted in isoprene rubber production workers; prevalence and degree were correlated with increasing length of service (IARC, 1994).

### 2.3.2 Consumer Exposure

There are no direct sales to consumers. Consequently, potential for consumer exposure will be negligible. Isoprene is used as a chemical intermediate primarily in the manufacture of polymers. Isoprene is also used for production of polymers used as food contact materials and was evaluated in this framework (Scientific Committee on Foods, 2000). Therefore exposure of consumers to this substance from the food contact materials need not be taken into account in the OECD framework. Isoprene is used in production of polymers used in paint resins, tires, footwear, moduled goods, adhesives, and motor oil viscosity improvers. An unknown percentage of unreacted monomers is present in the end-products. Possible migration rate is also unknown. The permission for use of isoprene rubber for food contact materials does not cover other consumer uses. The consumer exposure from other sources is unknown.

## 3 HUMAN HEALTH HAZARDS

### 3.1 Effects on Human Health

### 3.1.1 Toxicokinetics, Metabolism and Distribution

The metabolism and toxicokinetics of isoprene have been thoroughly reviewed by Watson *et al.* (2001) and Csanady and Filser (2001). Species differences in the endogenous production of isoprene have been reported. Isoprene metabolism as described by Michaelis-Menten kinetics has been shown to be saturable (Bond *et al.* 1991; Dahl *et al.*, 1987; and Peter *et al.*, 1987). The relevant studies are summarized below.

Studies in Animals

*In vivo Studies*

Isoprene is produced endogenously in both rats and mice. However, there are species differences in the rate of production. In studies by Peter *et al.* (1987, 1990) endogenous production of isoprene was calculated to be 1.9 and 0.4 µmol/hr/kg in rats and mice, respectively. When untreated rats or mice were kept in a closed exposure system the concentration of exhaled isoprene was measured to be 0.8 and 0.2 ppm, respectively. Under these particular conditions the metabolic rate for endogenously produced isoprene was estimated to be about 1.6 µmol/hr/kg in rats and 0.3 µmole/hr/kg in mice. However, Filser *et al.* (1996) reinvestigated the method of Peter *et al.* (1987)

and were unable to discriminate between the endogenous compounds acetone and isoprene. By using a different technique, Filser *et al.* (1996) detected smaller (several ppb) concentrations of endogenous isoprene production in rats compared to 0.6 ppm measured in humans.

In a pharmacokinetic study conducted by Peter *et al.* (1987), both rats and mice were exposed to 5 to 1000 ppm isoprene. The rate of metabolism was directly proportional to the exposure concentration at concentrations up to 300 ppm. However, both species exhibited saturation kinetics when exposed to isoprene at concentrations above 300 ppm. The maximal metabolic elimination rate in mice was determined to be 400 μmol/hr/kg or more. This shows the rate of metabolism in mice is about 2 or 3 times that found in rats (130 μmol/hr/kg).

Mice and hamsters showed more rapid and extensive metabolism to epoxide metabolites compared to rats and rabbits (Longo *et al.*, 1985). These epoxide metabolites are likely responsible for the toxic effects of isoprene and may explain why the mouse is more susceptible to isoprene toxicity.

In a study by Dahl *et al.* (1987), male F344 rats were exposed by nose-only inhalation for 6 hours to 8, 260, 1480, and 8200 ppm $^{14}$C-labeled isoprene. Increasing isoprene concentrations resulted in an increased amount of retained $^{14}$C metabolites; however, this was not a linear increase. The percentage of the inhaled isoprene that was metabolized decreased with increasing exposure concentration. The concentration of isoprene in the blood was below detection 1-2 minutes after termination of exposure to 8 or 260 ppm isoprene. About 75% of the total metabolites were excreted in urine, independent of inhaled isoprene concentration. After exposure to 8200 ppm, a larger percentage of the metabolites was excreted in feces than after exposure to lower concentrations. At one exposure concentration, 1480 ppm, metabolites were measured in the nose, lungs, liver, kidney, and fat, as well as in blood. A mutagenic metabolite, isoprene diepoxide, was tentatively identified in all tissues examined. Between 0.0018 and 0.031% of the inhaled $^{14}$C label was tentatively identified as isoprene diepoxide in blood. The relative amount of the metabolites present in blood was highest for low concentrations of inhaled isoprene and for shorter exposure durations. The appearance of metabolites in the respiratory tract after short exposure durations together with low blood concentrations of isoprene indicated that substantial metabolism of inhaled isoprene in the respiratory tract may occur. After an approximately 6-hr exposure to 1480 ppm isoprene, the concentration of reactive metabolites in blood reached a plateau. This demonstrates that the metabolism of isoprene is saturable.

In a study by Bond *et al.* (1991), male B6C3F1 mice were exposed to 20, 200 and 2000 ppm isoprene or $^{14}$C-isoprene for up to 6 hours. For all exposures, steady-state levels of isoprene were reached rapidly (i.e., within 15 to 30 minutes) after the onset of exposure. Hemoglobin adduct formation reached near-maximum between 200 and 2000 ppm which is consistent with the conclusion that pathways for metabolism of isoprene were saturated. The metabolism of isoprene was linearly related to exposure concentrations up to 200 ppm but decreased at 2000 ppm. Isoprene metabolites were present in blood after inhalation of isoprene at all concentrations studied.

*In vitro Studies*

Isoprene is metabolized by microsomal cytochrome P-450 dependent mono-oxygenases (Gervasi and Longo, 1990). Isoprene is metabolized by oxidation of either double bond resulting in the formation of two monoepoxides (i.e., 1,2-epoxy-2-methyl-3-butene and 3,4-epoxy-2-methyl-1-butene) as observed in liver microsomal preparations from rats, mice, rabbits, and hamsters. These monoepoxide metabolites may be hydrolyzed or conjugated with glutathione. The 3,4-epoxy-2-methyl-1-butene (minor pathway) may be further oxidized to the diepoxide, 2-methyl-1,2:3,4-diepoxide . The maximum metabolic velocity ($V_{max}$) of this oxidation reaction has been shown to be 6-fold higher in liver microsomes from mice and Syrian hamsters than from microsomes in rats

and rabbits (Bogaards *et al.*, 1996; DelMonte *et al.*, 1985; Gervasi and Longo, 1990; Longo et al., 1985; Small *et al.*, 1997; and Wistuba *et al.*, 1994).

Comparative studies with mammalian *in vitro* systems indicate that there are significant stereochemical and mechanistic differences among species (Small *et al.*, 1997). Enantiomers of the monoepoxide 2-(1-methylethenyl)oxirane were identified in liver microsome preparations from rats, mice, rabbits, dogs, monkeys, and humans. Rats preferentially formed (S)-2-(1-methylethenyl)oxirane compared with the (R)-enantiomer, whereas microsomes from dog, monkey, or male human preferentially formed (R)-2-(1-methylethenyl)oxirane. Metabolites from isoprene incubated with human female microsomes and with mouse and rabbit microsomes did not show enantioselectivity.

Studies in Humans

*In vivo Studies*

Isoprene is produced endogenously in humans, probably from mevalonic acid (Deneris *et al.*, 1984), a precursor of cholesterol biosynthesis. The endogenous production rate of isoprene was calculated to be 0.15 µmol/kg/hour (Hartmann and Kessler, 1990). Concentrations in human blood range between 15-70 nmol/L (mean = 37 nmol/L), but were <1 nmol/L in other animal species including rats, rabbits, and dogs (Cailleux *et al.*, 1992). Isoprene is also found in human breath at concentrations in the range of 10-30 nmol/L (Cailleux and Allain, 1989). The quantity of isoprene exhaled daily per individual was estimated to be 2-4 mg (Gelmont *et al.*, 1981).

In a preliminary report based on a study in 5 male and 1 female human volunteer exposed to isoprene via a closed spirometer system, the pharmacokinetics of isoprene in humans was reported to more closely resemble rats than mice (Hartmann and Kessler, 1990). These data were reported in more detail by Filser *et al.* (1996).

By generating data from human exposures at 0, 8, or 40 ppm isoprene, Filser et al. (1996) determined endogenous isoprene production for a 70 kg man to be 23.8 µmol/hr and the rate of metabolism to be 0.34 µmol/hr/kg. A toxicokinetic model predicted 3.4 mg of isoprene would be exhaled in a 24-hr period, which is in direct agreement with determinations made by other authors (Conkle *et al.*, 1975: 0.36 to 9.36 mg/24-hr; Gelmont *et al.*, 1981: 2 to 4 mg/24-hr). By using his toxicokinetic model to compare the data in mice (Bond *et al.*, 1991) and rats (Dahl *et al.*, 1987) with that obtained from humans (Filser *et al.*, 1996; Hartmann and Kessler, 1990), Filser *et al.* were able to show isoprene metabolism leading to production of both monoepoxides is up to 3.8 times faster in mice than in rats. At exposure concentrations up to 50 ppm, the rate of metabolism at steady state is 14 times faster in mice and approximately 8 times faster in rats than in humans.

*In vitro Studies*

The metabolism of isoprene and two isoprene monoepoxides was investigated with microsomes from cell lines expressing eight different human cytochrome P450 enzymes and with liver microsomes from humans, rats, and mice (Bogaards *et al.*, 1996). The single human enzymes were CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, CYP3A4. CYP2E1 showed the highest rates of formation for the two isoprene monoepoxides and was the only enzyme showing detectable formation of the isoprene diepoxide. Both isoprene monoepoxides were oxidized by CYP2E1 to the diepoxide at similar enzymatic rates. In order to determine the relative role of CYP2E1 in hepatic metabolism, isoprene as well as the two monoepoxides were incubated with a series of ten human liver microsomal preparations in the presence of the epoxide hydrolase inhibitor, cyclohexene oxide. Isoprene monoepoxide metabolism showed a significant correlation with CYP2E1 activity, determined as chlorzoxazone 6-hydroxylation. It was concluded that CYP2E1 is the major enzyme involved in hepatic metabolism of isoprene and the isoprene monoepoxides *in vitro*.

In order to investigate species differences with regard to the role of epoxide hydrolase in the metabolism of isoprene monoepoxides, the epoxidation of isoprene by human liver microsomes was compared to that of mouse and rat liver microsomes. The amounts of monoepoxides formed as a balance between epoxidation and hydrolysis, was measured in incubations with and without the epoxide hydrolase inhibitor cyclohexene oxide. Inhibition of epoxide hydrolase resulted in similar rates of monoepoxide formation in mouse, rat and man. However, without inhibitor the total amount of monoepoxides present at the end of the incubation period for mouse liver microsomes was twice as high as for rat and 15 times as high as for human liver microsomes (Bogaards *et al.*, 1996).

Conclusion

Clear differences exist in the toxicokinetics, metabolism and distribution of isoprene among species. Differences in metabolism and reactivity of the metabolites may contribute to the significant differences in toxicological response to isoprene observed between species.

### 3.1.2 Acute Toxicity

Studies in Animals

*Inhalation*

An acute inhalation exposure study was conducted by Shugaev (1969) to determine the concentrations of hydrocarbons such as isoprene in various tissues at lethal exposure concentrations. In this pre-GLP study minimal detail is provided. The $LC_{50}$ values reported in this study are as follows:

Rat $LC_{50}$ (4 hour) = 180 mg/L or 64,620 ppm (confidence limits 130 to 181 mg/L)

Mouse $LC_{50}$ (2 hour) = 157 mg/L or 56,363 ppm (confidence limits 129 to 252 mg/L)

An acute isoprene inhalation exposure study was also conducted by Gostinskii (1965). In this pre-GLP study conducted in mice, few study details are provided. However, the following $LC_{50}$ values are reported:

Female Mouse $LC_{50}$ (2 hour) = 148 mg/L or 53,121 ppm (confidence limits 144-153 mg/L)

Male Mouse $LC_{50}$ (2 hour) = 139 mg/L or 49,891 ppm (confidence limits 135-143 mg/L)

*Oral*

An acute oral toxicity study was conducted in Wistar rats. In this study, 15 animals per sex were administered isoprene in oil. The oral LD50 was determined to be 2043 to 2210 mg/kg. No other details were provided (Kimmerle and Solmecke, 1972).

Conclusion

The acute toxicity data on isoprene are limited. However, using a weight-of-evidence approach, the available data suggest that isoprene has a low order of acute toxicity in animals by the inhalation and oral routes of exposure.

### 3.1.3 Irritation

Studies in Animals

*Skin Irritation*

Although three studies are cited in the dossier, details are only available for the study conducted by Kimmerle and Solmecke (1972). In this skin irritation study, the skin of two New Zealand White rabbits was painted twice per day for 5 consecutive days with 100% isoprene. Reversible erythema was observed. Using a weight-of-evidence approach, the data suggest that isoprene has a low potential for skin irritation.

*Eye Irritation*

In a non-GLP study conducted by Mamedov (1979), isoprene was reported to cause eye irritation in rats. However, no study details are provided.

*Respiratory Tract Irritation*

A repeated dose inhalation study conducted in mice and rats exposed up to 7000 ppm (19,503 $mg/m^3$) isoprene for 6 hours/day, 5 days/week for 13 weeks showed no gross microscopic lesions in the respiratory tract of rats and female mice (Melnick *et al.*, 1994). In male mice degeneration of the olfactory epithelium was observed at 7000 ppm, but not at lower concentrations. The NOAEL in this study was 2200 ppm (6129 $mg/m^3$).

Studies in Humans

*Skin Irritation*

It has been reported that isoprene is irritating to the skin, eyes and mucous membranes (Lewis, 1996).

*Eye Irritation*

Vapors of isoprene have been reported to produce slight irritation of the eyes and upper respiratory tract. Liquid isoprene may irritate the eyes (Chemical Hazard Response Information System, 2001)

*Respiratory Tract Irritation*

Gostinskii (1965) reported that human volunteers experienced slight irritation of the upper respiratory tract at an isoprene concentration of 57 ppm (160 $mg/m^3$). However, the details in this pre GLP study are limited therefore, the reliability of the study cannot be assessed.

IARC reports that in isoprene rubber production workers, subtrophic and atrophic processes in the upper respiratory tract, catarrhal inflammation, and degeneration of the olfactory tract were observed. Prevalence and degree were correlated with increasing length of service (IARC, 1994).

Conclusion

Using a weight-of-evidence approach, isoprene appears to have a low potential for skin and respiratory tract irritation. However, the eye irritation potential of isoprene in animals or humans cannot be determined as no data are available for this endpoint.

### 3.1.4 Sensitization

No animal or human data are available for this endpoint.

### 3.1.5 Repeated Dose Toxicity

Studies in Animals

*Inhalation*

A 2-week repeated dose inhalation study was conducted in both mice and rats (Melnick *et al.*, 1990). In this study, F344 rats and B6C3F1 mice (20 animals/sex/group/species) were exposed by whole-body inhalation to isoprene at concentrations of 0, 438, 875, 1750, 3500, or 7000 ppm (i.e., 0; 1220; 2438; 4876; 9751; or 19,503 mg/$m^3$) for 6 hours/day, 5 days/week for two weeks. Ten animals/sex/group/species were used for clinical pathology evaluations after 4 exposures in rats or 5 exposures in mice. The remaining ten animals per group were used for histopathology at the end of the study. Body weights and clinical observations were recorded weekly. Necropsies were performed and major tissues/organs preserved. Histopathologic examinations were performed on the control and high exposure animals (7000 ppm), and lower dose groups until an apparent no-observed-effect level was found. The results for each species are described separately below.

In rats, no treatment-related changes in survival, body weight gain, clinical signs, hematologic or clinical chemistry parameters, organ weights, or incidences of gross or microscopic lesions were observed at any dose following the 2-week exposure. Thus, in this study, the NOAEL for rats was 7000 ppm (19,503 mg/$m^3$).

Exposure of the B6C3F1 mice to isoprene for 2-weeks did not produce mortality at any of the doses tested. A 15% reduction in body weight was observed in the 7000 ppm group of male mice. Exposure to isoprene did not cause reduction in mean body weight gain of female mice. Dose-related increases in mean liver weight/body weight ratios and decreases in relative thymus, spleen, and testis weights were observed in mice exposed to isoprene compared to controls. Organ weight changes were observed in both male and female mice.

In blood samples of mice exposed for 5 consecutive days to isoprene, mean red blood cell counts, hemoglobin concentrations, and volume of packed red cells were reduced in all exposure groups when compared to controls. These changes were not dose related nor accompanied by increases in reticulocyte counts or polychromatic erythrocytes. Similar hematologic changes were observed in male and female mice. The lack of exposure-related changes in serum chemistry parameters in mice indicates that the hepatic and renal systems were not adversely affected in this species after 5 days of exposure to isoprene.

Microscopic changes in the thymus, testes, nasal cavity and forestomach were observed in male mice following exposure to isoprene for 2 weeks. Microscopic forestomach lesions were also observed in exposed female mice. Thymic atrophy in male mice exposed to 7000 ppm was characterized by a decrease in cellularity of the cortex. Minimal testicular atrophy was observed in mice exposed to 7000 ppm isoprene. Diffuse liver changes consistent with highly glycogenated hepatocytes were observed to similar degrees in all dose groups of exposed male mice. Olfactory epithelial degeneration was observed at 1750, 3500, and 7000 ppm isoprene; the severity of the nasal lesions increased with increasing concentrations of isoprene.

Epithelial hyperplasia of the forestomach was seen in all groups of male and female mice exposed to isoprene. The severity of the lesion was relatively consistent throughout the exposure groups; a no observable-effect level for this lesion was not achieved at the exposure concentrations used in this study. The changes in spleen weights in mice exposed to isoprene were not associated with histopathological alterations in this organ. The significance of the observed changes in the liver of male mice but not female mice, is unclear.

In conclusion, this study demonstrated that there is a clear species difference in the susceptibility of rats and mice to isoprene exposure. In rats, there were no observable toxicological effects at any

dose following the 2-week exposure. The NOAEL for rats was 7000 ppm (19,503 mg/$m^3$). However, in mice, exposure to isoprene for 2 weeks induced changes in hematological parameters, body and organ weights and produced microscopic lesions in certain tissues at the lowest concentration tested, i.e., 438 ppm (1220 mg/$m^3$). Thus, in this study, the LOAEL for mice was 438 ppm (1220 mg/$m^3$).

A 13-week repeated dose whole-body inhalation study was conducted in mice and rats (Melnick *et al.*, 1994). In this study, F344 rats (10/sex/group) and B6C3F1 mice (10/sex/group) were exposed to 0, 70, 220, 700, 2200, and 7000 ppm (i.e., 0; 195; 613; 1950; 6129; 19,503 mg/$m^3$ ) isoprene, 6 hours/day, 5 days/week for 13 weeks. Body weights and clinical observations were recorded weekly. Blood samples were collected for clinical pathology evaluations on days 4, 24, and at the end of the study. Urine samples were collected from rats during week 12. After thirteen weeks of exposures, all rats and mice were sacrificed and evaluated histopathologically. Organ weights were also recorded.

In rats, no exposure-related effects were observed at any dose for survival, body weight gain, clinical signs of toxicity, hematology or clinical chemistry parameters, urinalysis, organ weights, or the incidence of gross or microscopic lesions.

In mice, there were no effects on survival, body weight gain, or clinical signs of toxicity. However, the male and female mice exposed to 700 ppm and higher showed hematologic effects indicative of a nonresponsive, macrocytic anemia at day 24 and after thirteen weeks. Focal epithelial hyperplasia of the forestomach was also observed in both males and females exposed to 700 ppm or higher. Degeneration of the olfactory epithelium was observed only in male mice at the highest concentration (i.e., 7000 ppm). Cytoplasmic vacuolization of hepatocytes due to glycogen accumulation was also observed in male mice at the two highest dose levels (i.e., 2200 and 7000 ppm). However, this effect was only statistically significant at the 7000 ppm dose level.

In conclusion, in this study, no toxicological effects were evident in rats exposed up to 7000 ppm (19,503 mg/$m^3$) for 13 weeks. However, in mice, hematological and histopathological changes were observed at exposures of 700 ppm (1950 mg/$m^3$) and higher. This 13-week repeated dose inhalation study, conducted as part of a 26-week carcinogenicity study, confirmed that mice are more susceptible to the effects of isoprene than rats.

Non-neoplastic effects were also observed following isoprene exposure in both the 26-week and the 104-week lifetime study conducted in mice and rats. These non-neoplastic effects are described below.

In the 26-week whole-body inhalation exposure study (Melnick et al., 1994), groups of 40 male B6C3F1 mice and Fischer 344 rats were exposed to 0, 70, 220, 700, 2200, or 7000 ppm (0; 195; 613; 1950; 6129; 19,503 mg/$m^3$) isoprene vapor by inhalation for 6 hours/day, 5 days/week for 6 months. At the end of the 26-week exposure period, 10 rats and 10 mice/group were sacrificed and evaluated. The remaining animals were allowed to recover for an additional 26 weeks without exposure at which time they were also sacrificed and evaluated. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically.

In rats, the only non-neoplastic effect observed following 26 weeks of exposure, was an increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7000 ppm (19,503 mg/$m^3$)isoprene group. No other gross or histopathological lesions in rats were attributed to isoprene exposures.

In mice, survival was reduced in the 7000 ppm group following the 26-week exposure period. In addition, near the end of the exposure period, abnormal posture and impaired hindlimb function were observed primarily in the 7000 ppm group; however, during the recovery period these clinical

signs subsided and affected animals gradually returned to a clinically normal state. Hindlimb grip strengths were significantly less in mice in the 220 ppm and higher exposure groups compared to controls. Hindlimb grip strengths remained lower than controls at day 2 of the recovery period. By 4 weeks postexposure, hindlimb grip strengths of exposed mice were generally similar to those of controls.

Although no treatment-related histopathological changes were detected in the lungs of isoprene-exposed mice at the end of the 26-week exposure period, an increased incidence of alveolar epithelial hyperplasia was observed in the 700 ppm and higher exposure groups following the 26-week recovery period. In addition, at the end of the 26-week exposure, focal hyperplasia of the forestomach epithelium, was observed in most mice in the 700, 2200 and 7000 ppm exposure groups. Following the 26-week recovery period, the incidence of forestomach hyperplasia was greater in the 700 ppm and higher exposure groups than in the controls.

Mild to minimal olfactory epithelial degeneration in the nasal cavity was also observed in all mice in the 7000 ppm exposure group after 26 weeks of exposure to isoprene. At the end of the 26-week recovery period, the incidence of mild to moderate olfactory epithelial degeneration was significantly elevated in the 220 ppm and higher exposure groups.

Exposure-related decreases in testis weight were observed in mice following 26 weeks of exposure to isoprene but not after the recovery period. Testicular atrophy was also observed in male mice exposed to 7000 ppm isoprene for 26 weeks but this effect was not observed after the recovery period.

Lastly, minimal degeneration of the spinal cord white matter was evident in mice exposed to 7000 ppm isoprene for 26 weeks; however, after the 26-week recovery period, the incidence of spinal cord degeneration was significantly increased in all exposure groups. Spinal cord degeneration most likely accounted for the hindlimb dysfunction discussed above. In a chronic oncogenicity study, $B6C3F_1$ mice were exposed to isoprene by inhalation for either 4 or 8 hours/day, 5 days/week for 20, 40 or 80 weeks (Placke *et al.*, 1996). Twelve groups of 50 male $B6C3F_1$ mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm (i.e., 0, 28, 195, 390, 780, 1950, 6129 $mg/m^3$) of isoprene vapor for 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period, leading to a total planned study length of 104 weeks. Female mice (50/group) were exposed to 0, 10, and 70 ppm of isoprene, 8 hours/day for 80 weeks and also held for observation through week 104. Selected groups of mice were removed at the end of 20 or 40 weeks of exposure, and were held in holding chambers for the duration of the 80 week exposure period. At the end of 80 weeks, all surviving animals were moved to a holding room through study week 104 and then necropsied beginning in study week 105.

With respect to nonneoplastic lesions, there were no apparent effects on motor function and no exposure-related lesions in the spinal cord at any concentration. This is in sharp contrast to what was observed in the NTP subchronic study where partial hindlimb paralysis and spinal cord degeneration was observed in mice exposed to 70 ppm (195 $mg/m^3$) for 6 months.

A chronic inhalation oncogenicity study was also conducted in rats. In this study, groups of 50 male and female F344/N rats were exposed to 220, 700, or 7000 ppm (613; 1950; 19,503 $mg/m^3$) isoprene by inhalation, 6 hours per day, 5 days per week, for 104 weeks (NTP, 1999). The survival of exposed males and females was similar to that of the chamber controls. Mean body weights of exposed male and female rats were similar to those of the chamber controls throughout the study.

Non-neoplastic findings in this study included renal tubule hyperplasia and splenic fibrosis. The incidence of renal tubule hyperplasia was significantly greater in males exposed to 7000 ppm (19,503 $mg/m^3$) isoprene than in the chamber controls. In addition, the severity of kidney nephropathy was slightly increased in 7000 ppm (19,503 $mg/m^3$) males when compared to chamber

controls. The incidences of splenic fibrosis in 700 ppm (1950 mg/m$^3$) and 7000 ppm (19,503 mg/m$^3$) males were significantly greater than that in the chamber control group.

Studies in Humans

Catarrhal inflammation, subtrophic and atrophic processes in the upper respiratory tract and deterioration of olfaction were noted in isoprene rubber production workers. Prevalence and degree were correlated with increasing length of service (IARC, 1994).Conclusion

High quality repeated dose studies demonstrate clear species differences between rats and mice in susceptibility to isoprene. For example, in rats, there were no observable toxicological effects at any dose following the 2-week repeated dose exposure. However, in mice, exposure to isoprene for 2 weeks induced changes in hematological parameters, body and organ weights and produced microscopic lesions in certain tissues at the lowest level tested, i.e., 438 ppm (1220 mg/m$^3$). Similarly, in the 13 week study, no toxicological effects were evident in rats exposed up to 7000 ppm (19,503 mg/m$^3$) isoprene for 13 weeks. However, in mice, hematological and histopathological changes were observed at exposures of 700 ppm (1950 mg/m$^3$) and higher. This 13-week repeated dose inhalation study confirmed the species difference between rats and mice in susceptibility to isoprene. The fact that mice are more susceptible to isoprene than rats was further demonstrated in both the 26-week and 104-week isoprene inhalation studies.

### 3.1.6 Mutagenicity

Isoprene has been tested for mutagenic activity in both *in vivo* and *in vitro* test systems. Anderson (2001) reviewed the genetic toxicity of isoprene. The critical studies are discussed below.

*In vivo Studies*

An in vivo Sister Chromatid Exchange (SCE) study and a Mammalian Bone Marrow Chromosomal Aberration study were conducted in B6C3F1 mice by Tice et al. (1988). In the SCE study, fifteen male B6C3F1 mice per group were exposed for 12 days, 6 hours/day to 0, 438, 1750, or 7000 ppm (0; 1220; 4876; 19,503 mg/m$^3$) of isoprene by inhalation. Positive control substances were not included in the study. The exposure regimen was 3 exposure days, 2 days off, 5 exposure days, 2 days off, then 4 exposure days. Exposure concentrations were monitored by gas chromatography. The animals were implanted with a BrdU tablet 1 hour before the 12th exposure. Two hours prior to sacrifice on the following day, the animals received an intraperitoneal injection of colchicine.

For analysis of SCE, 5 mice per exposure group were euthanized 24 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Twenty-five second-division metaphase cells were scored for SCEs from 4 mice/group.

For analysis of chromosomal aberrations, 10 mice per exposure group were killed 17-20 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Fifty first-division metaphase cells were scored for aberrations from 8 mice/group. Additionally, 100 randomly selected metaphase cells per slide were scored for replication history to provide data on cell generation time, a measure of cell proliferation kinetics. The percentage of cells in metaphase among 1000 cells/sample was used to calculate the mitotic index.

Exposure to isoprene for 6 hours/day at 0, 438, 1750, or 7000 ppm for 12 days induced a significant increase in the frequency of SCEs in bone marrow cells at all three dose levels (4.40 at 0 ppm, 14.84 at 438 ppm, 11.61 at 1750 ppm, and 13.98 at 7000 ppm). The increased SCE responses in the exposed groups were not statistically different from each other. The lack of significant difference in

SCEs among the three exposed groups suggests a saturation of the metabolic capacity of male mice to form reactive species. There were no significant clinical signs or mortality throughout the study.

In conclusion, in this study, isoprene was found to be genotoxic to bone marrow cells *in vivo* as indicated by the significant increase in the frequency of SCEs in bone marrow cells at all three dose levels. It was also found to be cytotoxic to mouse bone marrow as indicated by a significant lengthening of the bone marrow average generation time (AGTh) and the dose-dependent decline in the percentage of peripheral blood polychromatic erythrocytes (i.e., %PCE), a measure of the overall rate of erythropoiesis. In this study exposure to 0, 438, 1750 and 7000 ppm (0; 1220; 4876; 19,503 mg/m$^3$) isoprene resulted in an AGT (h) of 11.68, 12.98, 12.73 and 13.72, respectively. The %PCE following exposure to 0, 438, 1750 and 7000 ppm (0; 1220; 4876; 19,503 mg/m$^3$) isoprene was 3.91, 3.00, 2.87 and 1.64, respectively.

Exposure of mice to isoprene for 6 hours/day at concentrations of 0, 438, 1750, or 7000 ppm (0; 1220; 4876; 19,503 mg/m$^3$) for 12 days did not induce a statistically significant increase in the frequency of chromosomal aberrations in bone marrow cells (Tice et al.,1988). The incidence of bone marrow cells with chromosomal aberrations was slightly elevated in the exposed groups compared to the control but these increases were not statistically significant. Mitotic index data indicated no significant change in the percentage of bone marrow cells engaged in division, although the 7000 ppm group was slightly increased compared to the controls. Analysis of the average generation time showed a statistically significant lengthening of the cell cycle duration of proliferating cells in the 7000 ppm group.

In conclusion, although the incidence of bone marrow cells with chromosomal aberrations in male mice treated with isoprene for 12 days were slightly elevated at all dose groups compared to controls, none of the increases were statistically significant.

A Mammalian Erythrocyte Micronucleus Test was conducted in B6C3F1 mice by Tice et al. (1988). In this study, 15 male B6C3F1 mice per group were exposed by inhalation to isoprene at concentrations of 0, 438, 1750, and 7000 ppm (0; 1220; 4876; 19,503 mg/m$^3$), 6 hours/day for 12 days. Approximately 24 hours following the last exposure, peripheral blood samples were obtained from each animal by tail snip, air-dried immediately and fixed with methanol. One thousand polychromatic erythrocytes (PCEs) and 1000 normochromatic erythrocytes (NCEs) were scored per animal for frequency of micronucleated erythrocytes (MN). The percentage of PCEs in 1000 erythrocytes was also determined as a measure of isoprene-induced toxicity.

Exposure to isoprene induced a statistically significant increase in the frequency of MN-PCEs and NCEs in male mice at all exposure concentrations tested. There was also a dose-related decrease in the percentage of PCEs, a measure of the rate of erythropoiesis. There were no significant clinical signs or mortality throughout the study.

In conclusion, isoprene was genotoxic to mouse bone marrow *in vivo*. A decrease in the percentage of PCEs was also observed which suggests that erythropoiesis was also being suppressed.

A rat lung fibroblast Micronucleus Test was conducted in Fischer 344 rats by the National Toxicology Program (1997). Groups of 10 male and 10 female rats per group were exposed for 4 weeks to 0, 220, 700, or 7000 ppm (0; 613; 1950; 19,503 mg/m$^3$) isoprene by inhalation for a total of 17 to 19 exposures. The rats received at least two consecutive days of exposure prior to sacrifice and lung cell isolation. Lung fibroblasts were isolated and cultured in single-chamber slides for 72 hours. The slides were fixed and stained with acridine orange and 1000 binucleated cells on each of two slides per animal were scored. The number of mononucleated cells and micronuclei were recorded following a standard scoring criteria.

There were no statistically significant differences between the male or female exposed and control groups for micronucleated rat lung fibroblasts. There were no significant clinical signs or mortality during the exposure period.

In conclusion, isoprene was not genotoxic in this study. No significant increase in the frequency of micronucleated lung fibroblasts was observed in male and female rats exposed to isoprene for 4 weeks.

*In vitro Studies*

Isoprene was tested in an Ames assay in four strains of *Salmonella typhimurium* (i.e., TA98, TA100, TA1535, TA1537) with and without metabolic activation (Mortelmans et al., 1986). The preincubation modification of the Salmonella assay was used to test isoprene in these four different strains of Salmonella in the presence and absence of Aroclor 1254-induced rat and hamster liver S-9. Five dose levels plus control were tested (i.e., 0, 100, 333, 1000, 3333, and 10,000 μg/plate) with three plates per dose level. The high dose (10,000 μg/plate) was limited by toxicity. Concurrent positive controls were also tested with and without metabolic activation. The assay was repeated less than one week after completion of the initial test. All positive control substances produced at least a two-fold or three-fold increase in revertant colonies in their respective strains when compared with the vehicle controls. In this study, isoprene was not mutagenic in any of the four strains of Salmonella tested either in the presence or absence of Aroclor-induced rat or hamster liver S9. Concentrations of 0.25 to 25% isoprene monomer was subjected to Ames mutagenicity testing using vapor phase exposures (Huntington Life Sciences, Ltd., 2003a). To investigate possible species differences in metabolism, liver enzymatic preparations (S-9 and microsomes) were employed from uninduced B6C3F1 male mice. A positive control, vinyl chloride, provided evidence of both the mutagenic capabilities of the bacteria as well as activity of the liver enzymes. Salmonella strains TA1535, TA1537, TA98, and TA100 were employed as were E. coli WP2 uvrA (pKM101) bacteria. Criteria for a positive response included (a) evidence of dose-responsiveness, and (b) an increase in revertants of treated plates versus controls of 2 times for all strains except TA1535 and TA1537, which required a 3-fold increase. The maximal increase of revertant rates observed in isoprene-exposed bacteria was 1.7 for the TA1535 strain, and 1.6 in E. coli, both in the presence of S-9 activation. Vinyl chloride induced increases in revertant rates in these assays of 29- and 5.7-fold relative to negative controls, respectively. These results for isoprene indicate negligible mutagenic activity towards bacteria under the stated test conditions.

Isoprene was tested in an *in vitro* Sister Chromatid Exchange (SCE) assay in mammalian cells (Galloway et al., 1987). In this study, isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of SCEs both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and four doses of isoprene. The doses tested were 50, 160, 500, and 1600 μg/ml (without S9) and 160, 500, 1600, and 5000 μg/ml (with S9). A single flask per dose was used. All slides were scored blind and those from a single test were read by the same person. Fifty 2nd division metaphase cells were scored for frequency of SCEs/cell from each dose level. Isoprene was not genotoxic in this study as no increases in SCEs were noted in the cultured CHO cells treated with isoprene, with or without S9.

Isoprene was also tested in an *in vitro* Mammalian Chromosomal Aberration Test (Galloway et al., 1987). In this study, isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and three doses of isoprene. The doses tested were 1600, 3000 and 5000 μg/ml. A single flask per dose was used. All slides were scored blind and those from a single test were read by the same

person. Two hundred 1st-division metaphase cells were scored for chromosomal aberrations at each dose level.

The positive control substances produced statistically significant increases in the percentages of aberrant cells when tested with or without activation when compared to the vehicle controls. Isoprene was not genotoxic in this study as no increases in chromosomal aberrations were noted in cultured CHO cells treated with isoprene, with or without S9.

Conclusion

Isoprene was genotoxic to mouse bone marrow *in vivo*. Exposure of B6C3F1 mice to isoprene resulted in a statistically significant increase in sister chromatid exchanges and micronuclei in the bone marrow . However, isoprene did not produce an increase in micronucleated lung fibroblasts in exposed F344 rats. Isoprene was not genotoxic in any of the *in vitro* assays conducted, including those for bacterial mutation or for sister chromatid exchanges or chromosomal aberrations in exposed Chinese Hamster Ovary Cells.

### 3.1.7 Carcinogenicity

*In vivo* Studies - Inhalation

A. Melnick *et al.* (1994)

A 26-week inhalation exposure study was conducted with isoprene in F344 rats and B6C3F1 mice (Melnick *et al.*, 1994). In this study, groups of 40 male B6C3F1 mice and Fischer 344 rats were exposed to 0, 70, 220, 700, 2200, or 7000 ppm (i.e., 0; 195; 613; 1950; 6129; or 19,503 mg/m$^3$) isoprene vapor by inhalation for 6 hours/day, 5 days/week for 6 months. At the end of the 26-week exposure period, 10 rats and 10 mice/group were sacrificed and evaluated. The remaining animals were allowed to recover for an additional 26 weeks without exposure at which time they were also sacrificed and evaluated. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically.

Interstitial cell hyperplasia of the testis was observed in male rats after 26 weeks of exposure to 7000 ppm isoprene; following the 26-week recovery period, the only effect in rats was a marginal increase in benign testicular interstitial cell tumors in the 7000 ppm group. The survival of mice was reduced in the 7000 ppm group; early deaths were attributed to various neoplastic lesions and moribund sacrifices due to hindlimb paralysis. In male mice, incidences of malignant neoplastic lesions in the liver, lung, forestomach, and Harderian gland were significantly increased following the 26-week exposure and 26-week recovery periods at 700 ppm and higher exposures. However, in most cases, the carcinogenic effects of isoprene produced at 700 ppm (1950 mg/m$^3$) or 2200 ppm (6129 mg/m$^3$) were not very different from those at 7000 ppm (19,503 mg/m$^3$) (i.e., there was no clear dose-response). This is probably the result of metabolic saturation. Metabolic saturation has been shown to limit the production of epoxide intermediates at isoprene expoures greater than approximately 1500 ppm (4179 mg/m$^3$).

Isoprene was carcinogenic to the liver, lung, forestomach and Harderian gland of male mice after 26 weeks exposure and 26 weeks recovery. In contrast, the only effect observed in male rats was a marginally increased incidence of benign testicular adenomas at the highest exposure level (7000 ppm).

B. Placke *et al.* (1996)

In a chronic oncogenicity study, twelve groups of 50 male B6C3F$_1$ mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm (i.e., 0, 28, 195, 390, 780, 1950, or 6129 mg/m$^3$) isoprene vapor by

inhalation for 4 or 8 hours/day, 5 days/week for 20, 40 or 80 weeks. This was followed by a holding period, leading to a total planned study length of 104 weeks (Placke *et al.*, 1996). In this same study, female mice (50/group) were exposed to 0, 10, and 70 ppm of isoprene, 8 hours/day for 80 weeks and also held for observation through week 104. Selected groups of mice were removed at the end of 20 or 40 weeks of exposure, and were held in holding chambers for the duration of the 80 week exposure period. At the end of 80 weeks, all surviving animals were moved to a holding room through study week 104 and then necropsied beginning in study week 105. Complete histopathology examinations were performed on organs and tissues from all study animals. There was a concentration-related effect on survival with around 50% or fewer of those exposed to >280 ppm for 80 weeks surviving at 95 weeks.

Isoprene exposure produced an increase in histiocytic sarcomas and in neoplasms of the liver, lung, Harderian gland and forestomach of male mice. The incidence of hepatocellular adenomas and carcinomas were increased (significantly at exposure concentrations > 140 ppm), as were hemangiosarcomas and histiocytic sarcomas of the liver. Metastases of hepatocellular carcinomas to the lung were also more prominent in animals receiving higher exposures. Some of these primary and metastatic liver tumors appeared to be more anaplastic and aggressive in their growth as compared to the spontaneous liver tumors in controls.

Primary alveolar/bronchiolar adenomas and carcinomas were significantly increased in incidence in animals receiving 700 ppm for 80 weeks (i.e., 5600 ppm-weeks), 2200 ppm for 40 weeks (i.e., 88000 ppm-weeks), and 2200 ppm for 80 weeks (i.e., 176,000 ppm-weeks). Several lung carcinomas in exposed mice were locally invasive to the mediastinal and thoracic area, and in five cases metastasized to the liver. The lung was also the metastatic site for Harderian gland carcinomas, and two metastatic squamous cell carcinomas from the forestomach. Histiocytic sarcoma of the lung was also slightly more prevalent in isoprene-exposed mice than in controls.

The incidence of Harderian gland adenomas was significantly increased as the isoprene exposure concentration increased. Harderian gland carcinomas were not as numerous as adenomas but were present in the higher concentration groups. These carcinomas were diagnosed generally by the presence of foci or nodules of Harderian gland cells in the lung parenchyma or by evidence of Harderian cells in veins or lymph vessels leaving the tumor.

Squamous cells carcinomas of the stomach were present in six mice exposed to over 5600 ppm-weeks total exposure. Most were highly invasive locally and two metastasized to the lung. Squamous papillomas of the forestomach were only found in male mice in the highest exposure groups, i.e.: 56,000 ppm-weeks, 88,000 ppm-weeks, and 176,000 ppm-weeks.

Exposed mice also had a slightly increase incidence of hemangiosarcomas in the spleen and heart compared to controls. Cardiac hemangiosarcomas are very rare in B6C3F1 mice. A review of historical tumor incidence data shows no evidence of cardiac hemangisarcomas among 658 control B6C3F1 mice in recent 2-year inhalation studies.

Female mice were exposed to lower concentrations of isoprene than males. Neoplastic lesions that may have been exposure-related were hemangiosarcomas in the spleen, Harderian gland adenomas, and adenomas of the pituitary gland (pars distalis). Since the number of actual neoplasms in these organs was small in the female mice, historical incidences were considered (National Institute of Environmental Health Sciences, Research Triangle Park, NC). In 654 control mice from various inhalation studies, the number of splenic hemangiomas and/or hemangiosarcomas was four (0.61%), suggesting that the four hemangiosarcomas in the 70 ppm female (8%) may have been related to isoprene exposure. From the same data set, the historical incidence of Harderian adenomas was 22/661 (3.33%), with a range of 0 to 16% and the historical incidence of pituitary adenomas was 127/629 (20.19%), with a range of 2 to 44%. Thus, the relationship of these latter

two neoplasms to the isoprene exposure is only equivocal, considering the variability in the incidence of these lesions.

In this study, there appeared to be a well documented threshold for oncogenic effects following isoprene exposure in mice, which varied slightly by organ and tumor type. For male mice, the LOEL appeared to be 700 ppm for lung tumor and hemangiosarcoma, 280 ppm for malignant forestomach tumors and histiocytic sarcomas, 140 ppm for liver tumors, and 70 ppm for Harderian gland tumors. For female mice, the LOEL appeared to be 70 ppm for total non-liver, non-lung adenomas and possibly for hemangiosarcomas.

In summary, these results indicate that concentration, length of daily exposure, and weeks of exposure did not affect tumor incidence equivalently and total cumulative exposure was not sufficient for predicting oncogenic risk from isoprene exposure in mice. For example, it appears that exposure concentration has a greater impact on tumor rates than weeks of exposure (Cox *et al.*, 1996). In summary, the same cumulative exposure could be more or less damaging, depending upon how it was administered over time.

C. NTP (1999)

A chronic inhalation oncogenicity study was also conducted in rats. In this study, groups of 50 male and female F344/N rats were exposed to 220, 700, or 7000 ppm (613; 1950;19,503 mg/m3) isoprene by inhalation, 6 hours per day, 5 days per week, for 105-() weeks (NTP, 1999). The survival of exposed males and females was similar to that of the chamber controls. Mean body weights of exposed male and female rats were similar to those of the chamber controls throughout the study. Upon study termination, histopathological evaluations were performed on all major organ tissues from all study animals.

Exposure-related increases in the incidences of mammary gland fibroadenoma, and of fibroadenoma or carcinoma (combined), occurred in male rats in all exposure groups. Mammary gland fibroadenoma is considered to be a very rare tumor in male rats. The incidences of fibroadenoma in 7000 ppm males and all groups of exposed females were significantly greater than those in the chamber control groups. The incidences of fibroadenoma in all exposed groups of males and females and of multiple fibroadenoma in 7000 ppm males and in all groups of exposed females exceeded the historical control ranges.

The incidences of renal tubule adenoma in 700 and 7000 ppm males and the incidence of renal tubule hyperplasia in 7000 ppm males were significantly greater than those in the chamber controls. In addition, there was an exposure-related increase in the incidences of bilateral interstitial cell adenoma and of unilateral and bilateral interstitial cell adenoma (combined) of the testis in males exposed at 700 and 7000 ppm, the incidences being statistically significantly greater than those in the chamber controls. The incidences of interstitial cell adenoma in 700 and 7000 ppm males exceeded the historical control range. Several rare neoplasms including benign astrocytoma, malignant glioma, and malignant medulloblastoma, granular cell tumor and meningeal sarcoma were observed in the brain of exposed female rats. The neoplasms rarely occur in historical chamber controls. However, the fact that they are of different cell types makes it difficult to determine if they are truly exposure-related.

In summary, isoprene exposures were associated with increases in rates of benign tumors in the testes and kidney (male), and mammary gland (male and female). No significant increases were seen for malignant tumors in this study. For this reason, and the fact that brain tumors in females were of several distinct cell types, the overall level of evidence presented for the carcinogenicity of isoprene in rats is, at most, limited. In spite of this, the NTP concluded that under the conditions of this 2-year inhalation study, there was clear evidence of carcinogenic activity of isoprene in male F344/N rats based on increased incidences of mammary gland neoplasms, renal tubule adenoma,

and testicular adenoma. They also concluded that there was some evidence of carcinogenic activity of isoprene in female F344/N rats based on increased incidences and multiplicity of mammary gland fibroadenoma. A low incidence of rare brain neoplasms in exposed female rats may have been due to exposure to isoprene. In summary, based on the results of the carcinogenicity studies conducted in mice and rats, the NTP listed isoprene as "reasonably anticipated to be a human carcinogen" in the 9th Report on Carcinogens.

Conclusion

There is clear evidence of carcinogenicity of isoprene in mice. Isoprene produced exposure-related increases in the incidence of malignant neoplasms in the liver, lung, Harderian gland and forestomach of mice, as well as increases in the number of hemangiosarcomas and histiocytic sarcomas. In rats, on the other hand, there were no significant increases in the incidence of malignant tumors, but isoprene exposures were associated with increases in the rates of benign tumors in the testes and kidney (male) and mammary gland (male and female). Although single incidences of several rare brain neoplasms were observed in female rats, the fact that they were of several distinct cell types, makes it difficult to determine if they are truly exposure-related.

In summary, based on the results of the carcinogenicity studies conducted in mice and rats, the NTP listed isoprene as "reasonably anticipated to be a human carcinogen" in the 9th Report on Carcinogens. Based on their review, IARC (1999) has classified isoprene as a Group 2B carcinogen, i.e., possibly carcinogenic to humans.

### 3.1.8 Toxicity for Reproduction

The reproductive toxicity of isoprene has been reviewed by Anderson (2001). Relevant studies are discussed below.

Developmental Toxicity

In a well conducted NTP (National Toxicology Program, 1989) study, female Swiss CD-1 mice and Sprague-Dawley rats were exposed to 0, 280, 1400, or 7000 ppm (i.e., 0; 780; 3900; or 19,503 $mg/m^3$) isoprene for 6 hours/day, 7 days/week on gestational days 6-17 in mice or gestational days 6-19 in rats.

In rats, there was no adverse effect on the dam or offspring at any dose level and there was no increase in malformations or variations. A slight, but not statistically significant, increase in the incidence of reduced vertebral ossifications (centra) was noted at 7000 ppm. Thus, in rats 7000 ppm was the NOAEL for both maternal and developmental toxicity.

In mice, 7000 ppm isoprene significantly reduced maternal weight gain and uterine weight. Developmental toxicity was evident in mice as a statistically significant reduction in fetal bodyweight was observed at the 280 ppm level for female fetuses and at the 1400 ppm level for male fetuses. No embryotoxicity in the form of increased intrauterine death was present at any exposure level. Although there was no significant increase in the incidence of malformations, two fetuses with cleft palate were found, one in each of the two highest exposure groups (i.e., 1400 ppm and 7000 ppm). Cleft palate, however, is a common spontaneous finding in mice that occurs in response to maternal stress, and is not generally regarded as a manifestation of developmental toxicity in this species. Similarly, although increased incidences of variations (i.e., supernumerary ribs) were observed in the exposed groups, this skeletal variation is also considered as a secondary non-specific consequence of maternal toxicity.

In summary, in this study, 1400 ppm (3900 $mg/m^3$) was the NOAEL for maternal toxicity in mice. A NOAEL developmental toxicity could not be determined in this study because effects, i.e.,

reduction in fetal bodyweight, were observed at the lowest exposure concentration tested, i.e., 280 ppm (780 mg/$m^3$).

Reproductive Toxicity

No guideline reproductive studies have been conducted with isoprene. Histopathology of the reproductive organs was evaluated in a 13-week repeated dose inhalation study conducted in F344 rats and B6C3F1 mice at target concentrations up to 7000 ppm (19,503 mg/$m^3$)for 6 hours/day, 5 days/week for 13 weeks (Melnick et al., 1994). No exposure-related effects were observed in rats. In mice, testicular weight was reduced 35% in the 7000 ppm group, and morphological changes (seminiferous tubular atrophy) were detected in 2/10 mice.

Sperm motility and vaginal cytology were performed on all rats and mice exposed to 0, 70, 700, or 7000 ppm (0; 195; 1950; 19,503 mg/$m^3$) of isoprene in the 13-week study. Male mice in the 700 and 7000 ppm groups had 12% and 30% lower epididymal weights, 12% and 46% lower spermatid head counts, 12% and 46% lower sperm concentrations, and 6% and 23% reductions in sperm motility, respectively. The female mice exposed to 7000 ppm exhibited estrous cycle lengths significantly longer than the control group (4.8 vs. 4.2 days). Mice exhibited significant effects at 700 ppm (1950 mg/m3) or higher, including increased estrous cycle length and testicular atrophy, and decreased epididymal weight, sperm head count, sperm concentration, and sperm motility.

Conclusion

Inhalation exposure of pregnant rats and mice to isoprene at up to 7000 ppm (19,503 mg/$m^3$) produced maternal and developmental toxicity in mice but not in rats. In mice, maternal weight gain and uterine weight were significantly reduced at the highest dose (i.e., 7000 ppm). Significant reductions in fetal bodyweight were observed at the 280 ppm (780mg/$m^3$) dose level for female fetuses and at the 1400 ppm (3900 mg/$m^3$) level for male fetuses. Thus, in this study, 1400 ppm (3900 mg/$m^3$) was the NOAEL for maternal toxicity. A NOAEL for developmental toxicity could not be determined as effects were observed at the lowest exposure concentration tested, i.e., 280 ppm (780 mg/$m^3$). In less robust reproductive toxicity evaluations, conducted as part of an NTP subchronic inhalation toxicity study, no significant effects were observed after histopathological evaluations of reproductive organs in rats except slight changes in the testis at the highest exposure level (7000 ppm (19,503 mg/$m^3$)). However, significant effects were observed in mice exposed to isoprene concentrations of 700 ppm (1950 mg/$m^3$) and higher, including increased estrous cycle length and testicular atrophy as well as decreased epididymal weight. In addition, decreased sperm head count, sperm concentration, and sperm motility were also observed.

## 3.2 Initial Assessment for Human Health

The available data suggest that isoprene has a low potential for acute toxicity and for skin and respiratory tract irritation. Based on structure-activity relationships, the potential for dermal or respiratory tract sensitization is considered low. However, no data are available for this endpoint. The data for other endpoints suggest that there are marked species differences between the metabolism and toxicity of isoprene in mice versus rats. Mice metabolize isoprene more readily and are more susceptible to isoprene toxicity than rats. A physiological toxicokinetic model has been developed for inhaled isoprene in mice, rats, and humans, taking into account published or assumed kinetic parameters. On the basis of this model, at human exposure conditions (up to 50 ppm [140 mg/$m^3$]), rates of metabolism are about 14-times faster in mice and about 8-times faster in rats than in humans.

Four repeated-dose studies (i.e., 2-week, 13-week, 26-week, and 2-year) were conducted with isoprene. No observable toxicological effects were seen in rats following exposure to isoprene for 2

weeks at doses up to 7000 ppm. In mice, however, a 2-week exposure to isoprene induced changes in hematological parameters, body and organ weights and produced microscopic lesions in certain tissues at the lowest concentration tested, i.e. 438 ppm (1220 mg/m$^3$). In the 13-week study, no toxicological effects were evident in rats exposed up to 7000 ppm (19,503 mg/m$^3$). However, in mice, hematological and histopathological changes were observed at exposures of 700 ppm (1950 mg/m$^3$) and higher. In the 26-week study, the only treatment-related effects observed in rats were slight increases in the incidence of interstitial cell adenoma of the testis following exposure to 7000 ppm (19,503 mg/m$^3$). In mice, however, repeated exposure for 26 weeks to isoprene at concentrations of 700 ppm (1950 mg/m$^3$) and higher produced malignant neoplastic lesions in the liver, lung, forestomach and Harderian gland of male mice.

Non-neoplastic effects were also observed following isoprene exposure in both the 26-week and the 104-week lifetime study conducted in mice and rats. In rats, following 26 weeks of exposure, the only non-neoplastic effect observed was an increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7000 ppm (19,503 mg/m$^3$) group. However, in mice, a plethora of non-neoplastic effects were observed. In mice, survival was reduced in the 7000 ppm (19,503 mg/m$^3$) group following the 26-week exposure period. In addition, near the end of the exposure period, abnormal posture and impaired hindlimb function were observed primarily in the 7000 ppm (19,503 mg/m$^3$) group; however, during the recovery period these clinical signs subsided and affected animals gradually returned to a clinically normal state. Hindlimb grip strengths were significantly less in mice in the 220 ppm (613 mg/m$^3$) and higher exposure groups compared to controls. Hindlimb grip strengths remained lower than controls at day 2 of the recovery period. By 4 weeks postexposure, hindlimb grip strengths of exposed mice were generally similar to those of controls.

Although no treatment-related histopathological changes were detected in the lungs of isoprene-exposed mice at the end of the 26-week exposure period, an increased incidence of alveolar epithelial hyperplasia was observed in the 700 ppm (1950 mg/m$^3$) and higher exposure groups following the 26-week recovery period. In addition, at the end of the 26-week exposure, focal hyperplasia of the forestomach epithelium, was observed in most mice in the 700, 2200, and 7000 ppm (1950; 6129; 19,503 mg/m$^3$) exposure groups. Following the 26-week recovery period, the incidence of forestomach hyperplasia was greater in the 700 ppm (1950 mg/m$^3$) and higher exposure groups than in the controls.

Mild to minimal olfactory epithelial degeneration in the nasal cavity was also observed in all mice in the 7000 ppm (19,503 mg/m$^3$) exposure group after 26 weeks of exposure to isoprene. At the end of the 26-week recovery period, the incidence of mild to moderate olfactory epithelial degeneration was significantly elevated in the 220 ppm (613 mg/m$^3$) and higher exposure groups.

Exposure-related decreases in testis weight were observed in mice following 26 weeks of exposure to isoprene but not after the recovery period. Testicular atrophy was also observed in male mice exposed to 7000 ppm (19,503 mg/m$^3$) isoprene for 26 weeks but this effect was not observed after the recovery period.

Lastly, minimal degeneration of the spinal cord white matter was evident in mice exposed to 7000 ppm (19,503 mg/m$^3$) isoprene for 26 weeks; however, after the 26-week recovery period, the incidence of spinal cord degeneration was significantly increased in all exposure groups. Spinal cord degeneration most likely accounted for the hindlimb dysfunction discussed above.

In the chronic oncogenicity study conducted in B6C3F1 mice, no non-neoplastic lesions were observed. There were no apparent effects on motor function and no exposure-related lesions in the spinal cord at any concentration. This is in sharp contrast to what was observed in the NTP

subchronic study where partial hindlimb paralysis and spinal cord degeneration was observed in mice exposed to 70 ppm (195 mg/$m^3$) for 6 months.

In the chronic oncogenicity study conducted in rats, non-neoplastic findings included renal tubule hyperplasia and splenic fibrosis. The incidence of renal tubule hyperplasia was significantly greater in males exposed to 7000 ppm (19,503 mg/$m^3$) isoprene than in the chamber controls. In addition, the severity of kidney nephropathy was slightly increased in 7000 ppm (19,503 mg/$m^3$) males when compared to chamber controls. The incidences of splenic fibrosis in 700 ppm (1950 mg/$m^3$) and 7000 ppm (19,503 mg/$m^3$) males were significantly greater than that in the chamber control group.

Isoprene was tested for mutagenicity in a series of in vivo and in vitro studies. Isoprene was clearly genotoxic to mouse bone marrow in vivo. Exposure of B6C3F1 mice to isoprene resulted in a statistically significant increase in sister chromatid exchanges and bone marrow micronuclei. However, isoprene did not produce an increase in micronucleated lung fibroblasts in exposed F344 rats. These studies also demonstrate a clear species difference between mice and rats in susceptibility to isoprene. Isoprene was not genotoxic in any of the in vitro assays conducted.

Two-year inhalation carcinogenicity studies were conducted with isoprene in B6C3F1 mice and F344 rats. There is clear evidence of carcinogenicity of isoprene in mice. Isoprene produced exposure-related increases in the incidence of malignant neoplasms in the liver, lung, Harderian gland and forestomach of mice, as well as increases in the number of hemangiosarcomas and histiocytic sarcomas. In rats, there were no significant increases in the incidence of malignant tumors. Isoprene exposures in rats were associated with increases in the rates of benign tumors in the testes and kidney (male) and mammary gland (male and female). Although single incidences of several rare brain neoplasms were observed in female rats, the fact that they were of several distinct cell types makes it difficult to determine if they are truly exposure related. Based on the carcinogenicity studies conducted in mice and rats, the NTP listed isoprene "as reasonably anticipated to be a human carcinogen" in the 9th Report on Carcinogens and IARC has classified it as Group 2B, possibly carcinogenic to humans.

Isoprene did not produce any maternal or developmental toxicity in rats following exposure to concentrations as high as 7000 ppm (19,503 mg/$m^3$). However, both maternal and developmental toxicity were evident in mice. In mice, both maternal weight gain and uterine weight were significantly reduced at the highest dose (i.e., 7000 ppm (19,503 mg/$m^3$)). Significant reductions in fetal bodyweights were observed at the 280 ppm (780 mg/$m^3$) dose level for female fetuses and at the 1400 ppm (3900 mg/$m^3$) level for male fetuses. Thus, in this study, 1400 ppm (3900 mg/$m^3$) was the NOAEL for maternal toxicity. A NOAEL for developmental toxicity could not be determined as effects were observed at the lowest exposure concentration tested, i.e., 280 ppm (780 mg/$m^3$). Again, these studies clearly demonstrate that there is a species difference in sensitivity to isoprene between rats and mice.

In less robust reproductive toxicity evaluations, conducted as part of an NTP subchronic inhalation toxicity study, no significant effects were observed after histopathological evaluations of reproductive organs in rats except slight changes in the testis at the highest exposure level (7000 ppm (19,503 mg/$m^3$). However, significant effects were observed in mice exposed to isoprene concentrations of 700 ppm (1950 mg/$m^3$) and higher, including increased estrous cycle length and testicular atrophy as well as decreased epididymal weight. In addition, decreased sperm head count, sperm concentration, and sperm motility were also observed.

# 4 HAZARDS TO THE ENVIRONMENT

## 4.1 Aquatic Effects

Isoprene is expected to exhibit a moderate order of aquatic toxicity based on measured acute effects data that range from approximately 6 to 15 mg/L (Table 5).

Acute Toxicity Test Results

The measured isoprene freshwater fish (*Oncorhynchus mykiss*) 96-hour LC50 is 7.4 mg/L and invertebrate (*Daphnia magna*) 48-hour LC50 is 5.8 mg/L (Huntingdon Life Sciences Ltd., 2003b,c). The measured alga (*Pseudokirchneriella subcapitata*) 72-hour EC50 is 15 and >35 mg/L based on biomass and growth rate, respectively, while the 96-hour EC50 is 16 and >35 mg/L based on biomass and growth rate, respectively (Huntingdon Life Sciences Ltd., 2003d).

Chronic Toxicity Test Results

The measured alga (*Pseudokirchneriella subcapitata*) 72- and 96-hour NOEC for biomass is 1.7 mg/L, while the 72- and 96-hour NOEC for growth rate is 6.0 mg/L (Huntingdon Life Sciences Ltd., 2003d). Measured fish and invertebrate chronic data are not available.

**Table 5. Aquatic toxicity data for isoprene**

| Endpoint | Result (mg/L) |
|---|---|
| (*Oncorhynchus mykiss*) 96-hour $LC_{50}$ | 7.4 |
| (*Daphnia magna*) 48-hour $LC_{50}$ | 5.8 |
| (*Pseudokirchneriella subcapitata*) 72- and 96-hour $EbC_{50}$ | 16 |
| (*Pseudokirchneriella subcapitata*) 72- and 96-hour $ErC_{50}$ | 35 |
| (*Pseudokirchneriella subcapitata*) 72- and 96-hour NOECb | 1.7 |
| (*Pseudokirchneriella subcapitata*) 72- and 96-hour NOECr | 6.0 |

b biomassr growth rate

## 4.2 Terrestrial Effects

There are no experimental data available using standard testing procedures that can be used to assess the terrestrial hazard of isoprene. However, there is a calculated earthworm 14-day $LC_{50}$ value of 311 mg/kg soil (EPIWIN/ECOSAR, 1999). This value was calculated using a log $K_{ow}$ = 2.42.

## 4.3 Initial Assessment for the Environment

In the air, isoprene has the potential to rapidly degrade through indirect photolytic processes mediated primarily by hydroxyl radicals with a calculated degradation half-life of 1.2 hours depending on hydroxyl radical concentration. Aqueous photolysis and hydrolysis will not contribute to the transformation of isoprene in aquatic environments because it is either poorly or not susceptible to these reactions.

The photochemical ozone creation potential index for isoprene has been reported to range from 109.2 to 117.8, in comparison with a POCP index of 100 for ethylene, the reference substance.

Because of the relatively short half-life of isoprene in the atmosphere, its contribution to potential global warming can be considered minor.

Results of Mackay Level I distribution modeling at steady state show that isoprene will partition primarily to the air compartment (99.92%), with a negligible amount partitioning to water (0.06%) and soil (0.02%). Level III modeling indicates that at steady state, water is the primary compartment on a percentage basis when a default emission to this compartment is included in the calculations. Level III modeling may not be representative of the ultimate disposition of isoprene because default emission data (1000 kg/h/compartment) used in the model is not a representative rate of chemical discharge. However, concentrations in water are most likely very low because isoprene is quite volatile, and any volatilized substance will be quickly degraded in the atmosphere. When released primarily to the air compartment, the primary mode of removal would be via photodegradation. In spite of its water solubility, wet deposition of isoprene is not likely to play a significant role in its atmospheric fate because of rapid photodegradation. Volatilization to the air will contribute to the rapid loss of isoprene from aqueous and terrestrial habitats.

Isoprene has the potential to biodegrade to a significant extent based on results of ready biodegradation testing. However, microbial metabolism may not greatly contribute to its removal from the environment because of its potential to rapidly volatilize from aquatic and terrestrial media. Bioaccumulation of isoprene is unlikely based on a low potential to bioconcentrate.

Acute aquatic toxicity values for a fish and invertebrate are 7.4 (96hr-$LC_{50}$) and 5.8 (48hr-$EC_{50}$) mg/L, respectively. For algae, the lowest 96-hr effect value is 15 mg/L for biomass. Alga 96-hour NOEC values are 1.7 and 6.0 mg/L for biomass and growth rate, respectively.

There are no experimental terrestrial toxicity data available. However, a 14-day $LC_{50}$ value of 311 mg/kg soil has been calculated for an earthworm.

## 5 RECOMMENDATIONS

The chemical is currently of low priority for further work.

The chemical possesses properties indicating a hazard for human health (irritation, genotoxic, reproductive and developmental toxicity, carcinogenic) and the environment (fish, invertebrates, algae). Based on data presented by the Sponsor country, relating to production in one country which accounts for approximately 40% of global production and relating to the use pattern in one country, under normal manufacturing, formulation, industrial and consumer use of polymerized isoprene containing products, this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

## 6 REFERENCES

AIHA (American Industrial Hygiene Association) (2005). Workplace Environmental Exposure Guide (WEEL): Isoprene. AIHA, Fairfax, VA, USA.

Anderson D (2001). Genetic and reproductive toxicity of butadiene and isoprene. Chemico-Biological Interactions **135-136**, 65-85.

Bleasdale C, Small R, Watson W, Wilson J and Golding B (1996). Studies on the molecular toxicology of buta-1,3-diene and isoprene epoxides. Toxicol. **113**, 290-293.

Bogaards J, Venekamp J and van Bladeren P (1996). The biotransformation of isoprene and the two isoprene monoepoxides by human cytochrome P450 enzymes, compared to mouse and rat liver microsomes. Chemico-Biological Interactions **102**, 169-182.

Bond J, Bechtold W, Birnbaum L, Dahl A, Medinsky M, Sun J and Henderson R (1991). Disposition of inhaled isoprene in $B6C3F_1$ Mice. Toxicol. Applied Pharm. **107**, 494-503.

Brookhaven National Laboratory. Office of Science, U.S. Department of Energy Web site: http://www.face.bnl.gov/Modelling/isoprene.htm.

Budavari S (ed.) (1996). The Merck Index. 12th Edition. Merck & Co., Inc., Whitehouse Station, NJ, USA.

Cailleux A and Allain P, (1989). Isoprene and sleep. Life Sciences **44**, 1877-1880.

Cailleux A, Cogny M and Allain P (1992). Blood isoprene concentrations in humans and in some animal species. Biochemical Medicine and Metabolic Biology **47**, 157-160.

CHRIS: Chemical Hazard Response Information System (2001). U.S. Department of Transportation, U.S. Coast Guard. Washington, D.C. (Internet Version). Provided by Thomson MICROMEDEX, Greenwood Village, CO, USA.

CITI (Chemicals Inspection & Testing Institute) (1992). Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. Japan Chemical Industry Ecology-Toxicology & Information Center.

Columbia Encyclopedia, Sixth Edition (2005). Web site: http://www.encyclopedia.com/html/i1/isoprene.asp.

Conkle J, Camp B and Welch B (1975). Trace composition of human respiratory gas. Arch. Environ. Health **30**, 290-295.

Cox L, Bird M, and Griffis L (1996). Isoprene cancer risk and the time pattern of dose administration. Toxicology **113**, 263-272.

Csanady G and Filser J (2001). Toxicokinetics of inhaled and endogenous isoprene in mice, rats, and humans. Chemico-Biological Interactions **135-136**, 679-685.

Dahl A, Birnbaum L, Bond J, Gervasi P and Henderson R (1987). The fate of isoprene inhaled by rats: Comparison to butadiene. Toxicol. Applied Pharm. **89**, 237-248.

Del Monte M, Citti L and Gervasi P (1985). Isoprene metabolism by liver microsomal monooxygenases. Xenobiotica **15**, 591-597.

Deneris E, Stein R and Mead J (1984). *In vitro* biosynthesis of isoprene from mevalonate utilizing a rat liver cytosolic fraction. Biochem. Biophys. Res. Comm. **123** (2), 691-696.

Derwent R, Jenkin M and Saunders S (1996). Photochemical ozone creation potentials for a large number of reactive hydrocarbons under European conditions. Atmospheric Environ. **30,** 181-199.

Derwent R, Jenkin M, Saunders S and Pilling M (1998). Photochemical ozone creation potentials for organic compounds in Northwest Europe calculated with a master chemical mechanism. Atmospheric Environ. **32,** 2429-2441.

EPIWIN (1999). Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.

EPIWIN/ECOSAR (1999). Estimation Program Interface for Windows, version 3.04. ECOSAR Subroutine. Syracuse Research Corporation, Syracuse, NY, USA.

ExxonMobil Biomedical Sciences, Inc. 2004. Ready Biodegradability, Manometric Respirometry. Study #177294A. ExxonMobil Biomedical Sciences, Inc. Annandale, NJ, USA.

Fall, R. University of California, Los Angeles, CA, USA. Web site: http://www.colorado.edu/Chemistry/directory.dir/faculty.dir/biochem.dir/fall.dir/fallres.html.

Filser J, Csanady G, Denk B, Hartmann M, Kauffmann A, Kessler W, Kreuzer P, Putz C, Shen J, and Stei P (1996). Toxicokinetics of isoprene in rodents and humans. Toxicol. **113**, 278-287.

Galloway S, Armstrong M, Reuben C, Colman S, Brown B, Cannon C, Bloom A, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin B, Resnick M, Anderson B and Zeiger E (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environ. Mol. Mutagen. **10**, 1-175.

Gelmont D, Stein R and Mead J (1981). Isoprene - the main hydrocarbon in human breath. Biochem. Biophys. Res. Comm. **99**, 1456-1460.

Gervasi P and Longo V (1990). Metabolism and mutagenicity of isoprene. Environ. Health Perspect. **86**, 85-87.

Gould E (1959). Mechanism and Structure in Organic Chemistry. Holt, Reinhart and Winston, New York, NY, USA.

Gostinskii V (1965). Toxicity of isoprene and maximal safe concentration of the vapor in air. Federation Proceedings, Translation Supplement. **9**, 36-39. (English Translation).

Guenther A, Monson R and Fall R (1991). Isoprene and monoterpene emission rate variability: Observations with eucalyptus and emission rate algorithm development. J. Geophysical Res. **96**, 799-808.

Harris J (1982). Rate of Hydrolysis. In: Handbook of Chemical Property Estimation Methods. Environmental Behavior of Organic Compounds. Lyman W, Reehl W and Rosenblatt D (eds). McGraw-Hill, New York, NY, USA, **7**, 1-48.

Hartmann M and Kessler W (1990). Pharmacokinetics and endogenous production of isoprene in humans. Naunyn-Schmiedeberg's Arch Pharmacol 341(Suppl.), R13 (Abstract No. 50).

Howard P, Boethling R, Jarvis W, Meylan W and Michaenko E (1991). Handbook of Environmental Degradation Rates. Lewis Publishers, Inc., Chelsea, MI, USA, 439-440.

Huntingdon Life Sciences Ltd. (2003a). Ames Assay. Huntingdon Life Sciences Ltd., Cambridgeshire, England.

Huntingdon Life Sciences Ltd. (2003b). Acute Toxicity to Rainbow Trout (Semi-static exposure conditions). Project ID CSS 032. Huntingdon Life Sciences Ltd., Cambridgeshire, England.

Huntingdon Life Sciences Ltd. (2003c). Acute Toxicity to *Daphnia magna*. Project ID CSS 033. Huntingdon Life Sciences Ltd., Cambridgeshire, England.

Huntingdon Life Sciences Ltd. (2003d). Algal Growth Inhibition Assay, Project ID CSS 029. Huntingdon Life Sciences Ltd., Cambridgeshire, England.

IARC (1994). Monographs on the evaluation of the carcinogenic risk of chemicals to man. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, 1972-present (multi-volume work). **60**, 222-223.

IARC (1999). Monographs on the evaluation of the carcinogenic risk of chemicals to human. Lyon, France: World Health Organization, International Agency for Research on Cancer, 1972-present. (multi-volume work). **71**, 1015-1026.

International Labour Office (1983). Encyclopedia of Occupational Health and Safety. Vols. I&II. International Labour Office, Geneva, Switzerland, p. 1073.

Lacson J, Kaelin T and Yoneyama M (2005). Isoprene. SRI Abstract CEH. Web site: http://www.sriconsulting.com/CEH/Public/Reports/446.0000/.

Kimmerle G and Solmecke B (1972). Isopren-Akute Toxizitatsuntersuchungen. Bayer, AG, unveroffentlichter Bericht Nr. 3373. (Cited in IUCLID database; BG Chemie, 1991).

Klimisch H, Andreae M and Tillmann U (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicol. Pharm. **25**, 1-5.

Lewis, R.J. (2000). R.J. Sax's Dangerous Properties of Industrial Chemicals. 10th ed. Volumes 1-3. John Wiley & Sons, New York, NY, USA.

Longo V, Citti L and Gervasi P (1985). Hepatic microsomal metabolism of isoprene in various rodents. Toxicol. Letters **29**, 33-37.

Mackay D, Di Guardo A, Paterson S and Cowan C (1996). Evaluating the environmental fate of a variety of types of chemicals using the EQC model. Environ. Toxicol. Chem. **15**, 1627-1637.

Mackay D (1998). Level I Fugacity-Based Environmental Equilibrium Partitioning Model, Version 2.1 (16-bit). Environmental Modelling Centre, Trent University, Ontario, Canada.

Mamedov A (1979). Response of lymphoid tissue to single and multiple inhalation exposures to isoprene and some relevant integral indices. Gig. Tr. Prof. Zabol. 34-37.

McAuliffe C (1966). Solubility in water of Paraffin, Cycloparaffin, Olefin, Acetylene, Cycloolefin, and Aromatic Hydrocarbons. J. Physical Chem. **70**, 1267-1275.

Melnick R, Roycroft J, Chou B, Ragan H and Miller R (1990). Inhalation toxicology of isoprene in F344 and B6C3F1 mice following two-week exposures. Environ. Health Perspect. **86**, 93-98.

Melnick R, Sills R, Roycroft J, Chou B, Ragan H and Miller R (1994). Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. Cancer Res. **54**, 5333-5339.

Mendis S, Sobotka P and Euler D (1994). Pentane and isoprene in expired air from humans: gas-chromatographic analysis of single breath. Clinical Chem. **40**, 1485-1488.

Meylan W and Howard P (1993). Computer estimation of the atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone. Chemosphere **26**, 2293-2299.

Monson R, Harley P, Litvak M, Wildermuth M, Guenther A, Zimmerman P and Fall R (1994). Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. Oecologia **99**, 260-270.

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. Environ. Mutagen. **8** (Suppl. 7), 1-119.

Nartional Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 27709, Tumor Incidence in Control Animals by Route and Vehicle of Administration B6C3F1 Mice.

National Institute for Occupational Saftety and Health (NIOSH) (1989). National Occupational Exposure Survey. NIOSH, Washington, DC, USA.

National Toxicology Program (1989). Inhalation developmental toxicology studies: Teratology study of isoprene in mice and rats. TER88045; NTIS#DE89008095.

National Toxicology Program (1999). Toxicology and Carcinogenesis studies of isoprene (CAS No. 78-79-5) in F344/N rats (Inhalation Studies). Report No. TR-486.O'Neil MJ, Smith A, Heckelman PE and Budavari S (eds.) (2001). The Merck Index – An Encyclopedia of Chemicals, Drugs, and Biologicals. Thirteenth Edition. Merck Research Laboratories, Merck & Co., Inc. Whitehouse Station, NJ, USA.

Peter H, Wiegand H, Bolt H, Greim H, Walter G, Berg M and Filser J (1987). Pharmacokinetics of isoprene in mice and rats. Toxicol. Letters **36**, 9-14.

Peter H, Wiegand H, Filser G, Bolt H, Laib R (1990). Inhalation pharmacokinetics of isoprene in rats and mice. Environ. Health Perspect. **86**, 89-92.

Placke M, Griffis L, Bird M, Bus J, Persing R and Cox L Jr. (1996). Chronic inhalation oncogenicity study of isoprene in B6C3F1 mice. Toxicol. **113**, 253-262.

RTECS (The Registry of Toxic Effects of Chemical Substances). Web site: http://www.cdc.gov/niosh/rtecs/nt3d9988.html.

Scientific Committee on Foods (2000). European Commission, Health and Consumer Protection Directorate-General. SCF/CS/PM/GEN/M83 13 November 2000.

Shugaev B (1969). Concentrations of hydrocarbons in tissues as a measure of toxicity. Arch. Environ. Health **18**, 878-882.

Small R, Golding B and Watson W (1997). Species differences in the stereochemistry of the metabolism of isoprene *in vitro*. Xenobiotica **2**, 1155-1164.

Song J*, Vizuete W*, Kimura Y*, Allen D* and Jeffries H**. Comparison of observed and modeled isoprene concentrations in southeast Texas during the Texas Air Quality Study. *Center for Energy and Environmental Resources, University of Texas (TX, USA); **Department of Environmental Sciences and Engineering, University of North Carolina (NC, USA). Web site: http://www.tceq.state.tx.us/assets/public/policy/epi/sip/sipdocs/2004-05-HGB/xml=http://www.tnrcc.state.tx.us/cgi-bin/texis/webinator/search/pdfhi.txt?query=isoprene+emissions+data&pr=publicProd&prox=page&

rorder=500&rprox=500&rdfreq=500&rwfreq=500&rlead=500&sufs=0&order=r&cq=&id=42377d667.

SRI International (2000). SRI Consulting, Menlo Park, CA, USA.

Taalman R (1996). Isoprene: background and issues. Toxicology **113**, 242 – 246.

Tice R, Boucher R, Luke C, Paquette D, Melnick R and Shelby M (1988). Chloroprene and isoprene: cytogenetic studies in mice. Mutagen. **3** (2), 141-146.

USITC (United States International Trade Commission) (1995). Washington, DC, USA.

Watson W, Cottrell L, Zhang D, and Golding B (2001). Metabolism and molecular toxicology of isoprene. Chemico-Biological Interactions **135-136**, 233-238.

Wistuba D, Weigand K and Peter H (1994). Stereoselectivity of *in vitro* isoprene metabolism. Chem. Res. Toxicol. **7**, 336-343.

Zepp R and Cline D (1977). Rates of direct photolysis in the aqueous environment. Environ. Sci. Technol. **11**, 359-366.

Zwolinski BJ and Wilhoit RC (1971). Handbook of Vapor Pressures and Heats of Vaporization of Hydrocarbons and Related Compounds. AP144-TRC101. Thermodynamics Research Center, College Station, TX, USA.

# S I D S

# Dossier

| | | |
|---|---|---|
| **Existing Chemical** | **:** | ID: 78-79-5 |
| **CAS No.** | **:** | 78-79-5 |
| **EINECS Name** | **:** | Isoprene |
| **EC No.** | **:** | 201-143-3 |
| **TSCA Name** | **:** | 1,3-Butadiene, 2-methyl- |
| **Molecular Formula** | **:** | C5H8 |

**Producer related part**

| | | |
|---|---|---|
| **Company** | **:** | ExxonMobil Biomedical Sciences Inc. |
| **Creation date** | **:** | 26.03.2003 |

**Substance related part**

| | | |
|---|---|---|
| **Company** | **:** | ExxonMobil Biomedical Sciences Inc. |
| **Creation date** | **:** | 26.03.2003 |
| **Status** | **:** | |
| **Memo** | **:** | American Chemistry Council Olefins Panel for the ICCA HPV initiative |
| **Printing date** | **:** | 29.07.2005 |
| **Revision date** | **:** | |
| **Date of last update** | **:** | 29.07.2005 |
| **Number of pages** | **:** | |
| **Chapter (profile)** | **:** | Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10 |
| **Reliability (profile)** | **:** | Reliability: without reliability, 1, 2, 3, 4 |
| **Flags (profile)** | **:** | Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS |

### 1.0.1 APPLICANT AND COMPANY INFORMATION

### 1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

### 1.0.3 IDENTITY OF RECIPIENTS

### 1.0.4 DETAILS ON CATEGORY/TEMPLATE

### 1.1.0 SUBSTANCE IDENTIFICATION

### 1.1.1 GENERAL SUBSTANCE INFORMATION

**Purity type** :
**Substance type** : Organic
**Physical status** : Liquid
**Purity** : ca. 99 % w/w
**Colour** :
**Odour** :

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.08.1997

### 1.1.2 SPECTRA

### 1.2 SYNONYMS AND TRADENAMES

**2-methyl-1,3-butadiene**

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.08.1997

**2-Methylbutadiene**

23.02.2004

**3-Methyl-1,3-Butadiene**

23.02.2004

**Beta-Methylbivinyl**

23.02.2004

**Hemiterpene**

23.02.2004

**Isoprene**

23.02.2004

**Methylbivinyl**

23.02.2004

## 1.3 IMPURITIES

**Purity** :
**CAS-No** : 138-86-3
**EC-No** : 205-341-0
**EINECS-Name** : isoprene dimer (dipentene)
**Molecular formula** :
**Value** : <= .5 % w/w

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.08.1997

## 1.4 ADDITIVES

**Purity type** :
**CAS-No** : 98-29-3
**EC-No** : 202-653-9
**EINECS-Name** : p-tert.-butyl catechol
**Molecular formula** :
**Value** :
**Function of additive** :

**Remark** : Isoprene contains 150-250 ppm p-tert. butyl catechol as inhibitor/stabilizer.
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.08.1997

## 1.5 TOTAL QUANTITY

**Remark** : Isoprene world consumption in 2000 was 579,000 metric tons, of which approximately 96% was consumed in the country of manufacture (SRI International, 2000). In the United States, isoprene production in 1995 was approximately 619 million lb (281,000 Mg [metric tons]) (USITC, 1995).

29.07.2005 (71) (75)

## 1.6.1 LABELLING

**Symbols** : F+, , ,
**Nota** : , ,

| | | |
|---|---|---|
| **R-Phrases** | : | (12) Extremely flammable<br>(45) May cause cancer<br>(52/53) Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment |
| **S-Phrases** | : | (45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)<br>(53) Avoid exposure - obtain special instructions before use<br>(61) Avoid release to the environment. Refer to special instructions/Safety data sets |
| **Remark** | : | 45 -12 - 68 - 52/53 |

29.07.2005

### 1.6.2 CLASSIFICATION

| | | |
|---|---|---|
| **Classified** | : | |
| **Class of danger** | : | |
| **R-Phrases** | : | (12) Extremely flammable<br>(45) May cause cancer<br>(52/53) Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment |
| **Specific limits** | : | |
| **Remark** | : | F+; 12 - (Carc. Cat. 2) - 45 - (Muta. Cat. 3) - 68 - 52/53 |

29.07.2005

### 1.6.3 PACKAGING

## 1.7 USE PATTERN

| | | |
|---|---|---|
| **Type of use** | : | Industrial |
| **Category** | : | Polymers industry |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |

11.01.2005

| | | |
|---|---|---|
| **Type of use** | : | Industrial |
| **Category** | : | Polymers industry |
| **Remark** | : | Isoprene is a chemical intermediate that is used in the synthesis of elastomers, such as poly(cis-1,4-isoprene), butyl-rubber, and coblock polymers. |

11.01.2005

| | | |
|---|---|---|
| **Type of use** | : | Industrial |
| **Category** | : | Polymers industry |
| **Remark** | : | Isoprene is used as a chemical intermediate to manufacture primarily polymers, which occurs in closed production systems. Greater than 95% of high-purity isoprene is used as a monomer to manufacture elastomers such as polyisoprene, styrenic thermoplastic elastomer block copolymers (styrene-isoprene-styrene [SIS]), and butyl rubber. The remaining amount |

of isoprene is used to manufacture specialty chemicals, including vitamins, pharmaceuticals, flavorings and perfumes, and epoxy hardeners.

11.01.2005

### 1.7.1 DETAILED USE PATTERN

### 1.7.2 METHODS OF MANUFACTURE

**Remark** : Isoprene is obtained by extractive distillation from an isoprene concentrate stream produced by the ethylene production process. In the pyrolysis furnaces of the ethylene production process, paraffinic feedstocks such as ethane, propane, naphthas or gas oils, are subjected to high temperatures in the presence of steam. These conditions result in the partial conversion or cracking of the hydrocarbon feedstock components and formation of unsaturated hydrocarbons. Ethylene and propylene are the primary products, but other olefins, diolefins, aromatics and cyclics are also produced, including a relatively small amount of isoprene. The ethylene process compresses and separates the pyrolysis furnace effluent into product streams. Isoprene produced in the cracking furnace is contained in one of these product streams, the pyrolysis gasoline.

Pyrolysis gasoline is a complex hydrocarbon mixture, consisting predominately of carbon number five (C5+) and higher hydrocarbon components. Distillation of pyrolysis gasoline produces a pyrolysis C5 stream. This stream is also a complex mixture, and consists predominately of the carbon number 5 olefins and diolefins that were produced in the ethylene process cracking furnaces. The stream also includes n-pentane and iso-pentane, which may result largely due to the unconverted pentanes in the ethylene process feedstock. Further processing of the C5 stream results in an isoprene concentrate stream that is separated from the Pyrolysis C5 by a series of distillation and "heat soak" operations. The "heat soak" is used to convert cyclopentadiene to its dimer (dicyclopentadiene) in order to facilitate isolation of the isoprene concentrate. Isoprene concentrate thus produced from the pyrolysis C5 stream has a typical isoprene content of 40%. This concentrate is then processed in an extractive distillation unit that uses a solvent such as acetonitrile to facilitate isolation of the contained isoprene as a 99% purity product.

Isolation of isoprene from the ethylene process co product streams as described above is the primary source of isoprene. "Only this method of production is practiced in the United States and Western Europe. On-purpose synthetic routes to isoprene are also used commercially, including dehydrogenation of isoamylene and isopentane (capacity in Russia) and reaction of isobutylene with formaldehyde (Russia and Japan)."

**Source** : American Chemistry Council, Olefins Panel

29.07.2005 (40)

### 1.8 REGULATORY MEASURES

### 1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

**Remark** : none established
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.08.1997

**1.8.2 ACCEPTABLE RESIDUES LEVELS**

**1.8.3 WATER POLLUTION**

**1.8.4 MAJOR ACCIDENT HAZARDS**

**1.8.5 AIR POLLUTION**

**1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES**

**1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS**

**1.9.2 COMPONENTS**

**1.10 SOURCE OF EXPOSURE**

**Remark** : Exposure during polymer processing very low under good industrial hygiene and safety at work conditions.

Exposure caused by migration of residue monomer regarded as negligible.
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.08.1997

**Remark** : Exposure to isoprene may occur at workplaces where it is manufactured. Based on physical properties, the primary workplace exposure would be by inhalation. No consumer exposure is foreseen because there are no direct sales to consumers.

23.02.2004

**Remark** : Isoprene is a petrochemical that is used as a chemical intermediate in contained systems. Potential occupational exposure to isoprene through inhalation and dermal contact could occur at workplaces where isoprene or synthetic rubber is produced or used.

23.02.2004

**Remark** : The National Institute for Occupational Safety and Health (NIOSH)

estimated that 3,654 workers (578 of these are female) were potentially exposed to isoprene in the US.

**Source** : National Institute for Occupational Saftety and Health (NIOSH) (1989). National Occupational Exposure Survey. NIOSH, Washington, DC, USA.

23.02.2004

## 1.11 ADDITIONAL REMARKS

## 1.12 LAST LITERATURE SEARCH

**Type of search** : Internal and External
**Chapters covered** :
**Date of search** : 04.05.2004

**Remark** : Aquire (1992 - present)
Biodegradation Data (BIODEG) (1992 - present)
Biodegradation Bibliographic References (BIOLOG)(1992 - present)
Biological Abstracts - BIOSIS (1969 - present)
Cancerlit (1975 - present)
EMBASE (1974 - present)
Enviroline (1970 - present)
Environmental Bibliography (1974 - present)
Gene-Tox (1992 - present)
Medline (1960 - present)
National Technical Information Service (NTIS)(1964 - present)
NIOSH (1973 - present)
PASCAL (1973 - present)
Pollution Abstracts (1970 - present)
TERRETOX (1992 - present)
TSCATS (1977 - present)
Toxfile (1965 - present)

29.07.2005

## 1.13 REVIEWS

**Memo** : Metabolism

**Remark** : Isoprene is metabolized in mammals in processes that involve epoxidation by cytochrome P450-dependent monooxygenases to the isomeric mono-epoxides, (1-methylethenyl)-oxirane and 2-ethenyl-2-methyloxirane Further metabolism of the mono-epoxides to mutagenic isoprene di-epoxides can also occur. The oxidations to the mono- and di-epoxides occur enantioselectively and diastereoselectively. The mono- epoxides are hydrolyzed enantioselectively to vicinal diols under catalysis by epoxide hydrolase. 2-Ethenyl-2-methyloxirane is also readily hydrolyzed non-enzymatically. Because of the stereochemical possibilities for metabolites, the metabolism of isoprene is complex. The metabolism of isoprene by liver microsomes in vitro from a range of species including rat, mouse, and human shows significant differences between species, strains and gender in respect of the diastereoselectivity and enantioselectivity of the metabolic oxidation and hydrolysis reactions. The impact of the extra methyl in isoprene on di-epoxide reactivity also appears to be critically important for the resulting biological effects. Isoprene di-epoxides may exhibit a lower cross-linking potential in vivo compared to butadiene di-epoxides. Differences in metabolism and reactivity of metabolites may be factors

contributing to the significant differences in toxicological response to isoprene observed between species.

29.07.2005 (76)

**Memo** : Reproduction

**Remark** : This paper provides a comprehensive review of the genetic toxicology and the reproductive/developmental effects of butadiene and isoprene.

29.07.2005 (1)

## 2.1 MELTING POINT

**Value** : = -145.9 °C
**Sublimation** :
**Method** : other: not specified
**Year** :
**GLP** : no data
**Test substance** : other TS: Isoprene

**Test substance** : Isoprene purity is unknown.
**Reliability** : (2) valid with restrictions
The Merck Index is a chemical handbook that is a peer reviewed publication. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
**Flag** : Critical study for SIDS endpoint
08.04.2003 (57)

**Value** : = -118.9 °C
**Sublimation** :
**Method** : other: calculated
**Year** :
**GLP** :
**Test substance** : other TS: Isoprene

**Method** : The calculated value was determined using MPBPWIN version 1.40, a subroutine within the computer program EPIWIN version 3.04.
Melting Point estimations performed by MPBPWIN are based on the average result of the calculation methods of K. Joback and Gold and Ogle. Joback's Method is described in Joback, K.G. 1982. A Unified Approach to Physical Property Estimation Using Multivariate Statistical Techniques. In The Properties of Gases and Liquids. Fourth Edition. 1987. R.C. Reid, J.M. Prausnitz and B.E. Poling, Eds.
The Gold and Ogle Method simply uses the formula
Tm = 0.5839Tb, where Tm is the melting point in Kelvin and Tb is the boiling point in Kelvin.
**Reliability** : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.
31.03.2003 (18)

## 2.2 BOILING POINT

**Value** : = 34 °C at
**Decomposition** :
**Method** : other: not specified
**Year** :
**GLP** : no data
**Test substance** : other TS: Isoprene

**Test substance** : Isoprene purity is unknown.
**Reliability** : (2) valid with restrictions
The Merck Index is a chemical handbook that is a peer reviewed publication. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
**Flag** : Critical study for SIDS endpoint

31.03.2003 (57)

**Value** : = 35 °C at
**Decomposition** :
**Method** : other: calculated
**Year** :
**GLP** :
**Test substance** : other TS: Isoprene

**Method** : The calculated value was determined using MPBPWIN version 1.40, a subroutine within the computer program EPIWIN version 3.04.
Boiling Point estimations performed by MPBPWIN are based on the calculation method of S. Stein and R. Brown in "Estimation of Normal Boiling Points from Group Contributions". 1994. J. Chem. Inf. Comput. Sci. 34: 581-587.
**Reliability** : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.

31.03.2003 (18)

## 2.3 DENSITY

**Type** : density
**Value** : = .681 g/cm³ at 20 °C
**Method** :
**Year** :
**GLP** : no data
**Test substance** : other TS: Isoprene

**Test substance** : Isoprene purity is unknown.
**Reliability** : (2) valid with restrictions
The Merk Index, an encyclopedia of chemicals, drugs, and biologicals, is a peer reviewed publication. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
**Flag** : Critical study for SIDS endpoint

29.07.2005 (57)

### 2.3.1 GRANULOMETRY

## 2.4 VAPOUR PRESSURE

**Value** : = 733.3 hPa at 25 °C
**Decomposition** :
**Method** :
**Year** :
**GLP** : no data
**Test substance** : other TS: Isoprene

**Method** : Method not specified.
**Test substance** : Isoprene purity is unknown.
**Reliability** : (2) valid with restrictions
The Handbook of Vapor Pressures and Heats of Vaporization of Hydrocarbons and Related Compounds is a peer reviewed publication.

This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.

**Flag** : Critical study for SIDS endpoint
31.03.2003 (79)

**Value** : = 734.6 hPa at 25 °C
**Decomposition** :
**Method** : other (calculated)
**Year** :
**GLP** :
**Test substance** : other TS: Isoprene

**Method** : Calculated values using MPBPWIN version 1.40, a subroutine of the computer program EPIWIN version 3.04
Vapor Pressure estimations performed by MPBPWIN are based on the average result of the calculation methods of Antoine and Grain. Both methods use boiling point for the calculation.
The Antoine Method is described in the Handbook of Chemical Property Estimation. Chapter 14. W.J. Lyman, W.F. Reehl and D.H. Rosenblatt, Eds. Washington, D.C.: American Chemical Society. 1990.
A modified Grain Method is described on page 31 of Neely and Blau's Environmental Exposure from Chemicals, Volume 1, CRC Press. 1985.
**Reliability** : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.
31.03.2003 (18)

## 2.5 PARTITION COEFFICIENT

**Partition coefficient** :
**Log pow** : = 2.42 at °C
**pH value** :
**Method** :
**Year** :
**GLP** : no data
**Test substance** : other TS: Isoprene

**Test substance** : Isoprene purity is unknown.
**Reliability** : (2) valid with restrictions
The data are cited in the Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
**Flag** : Critical study for SIDS endpoint
29.07.2005 (9)

**Partition coefficient** :
**Log pow** : = 2.42 at °C
**pH value** :
**Method** :
**Year** :
**GLP** : no data
**Test substance** : other TS: Isoprene

**Method** : Method not specified.
**Test substance** : Isoprene purity is unknown.
**Reliability** : (2) valid with restrictions

The value is cited in the EPIWIN experimental database (SRC Physprop Database) for isoprene. Although the original reference was not retrieved and reviewed for quality, this robust summary has a reliability rating of 2 because the data are from a peer reviewed database.

29.07.2005 (18)

| | | |
|---|---|---|
| **Partition coefficient** | : | |
| **Log pow** | : | = 2.58 at °C |
| **pH value** | : | |
| **Method** | : | other (calculated) |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | other TS: Isoprene |

**Method** : Calculated values using KOWWIN version 1.65, a subroutine of the computer program EPIWIN version 3.04
Octanol / Water Partition Coefficient estimations performed by KOWWIN are based on an atom/fragment contribution method of W. Meylan and P. Howard in "Atom/fragment contribution method for estimating octanol-water partition coefficients". 1995. J. Pharm. Sci. 84:83-92.

**Reliability** : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.

29.07.2005 (18)

### 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

| | | |
|---|---|---|
| **Solubility in** | : | Water |
| **Value** | : | = 642 mg/l at 25 °C |
| **pH value** | : | |
| **concentration** | : | at °C |
| **Temperature effects** | : | |
| **Examine different pol.** | : | |
| **PKa** | : | at 25 °C |
| **Description** | : | |
| **Stable** | : | |
| **Deg. product** | : | |
| **Method** | : | other: measured |
| **Year** | : | |
| **GLP** | : | no |
| **Test substance** | : | other TS: Isoprene |

**Method** : From 10 to 20 ml of test substance was added to 200 ml of distilled water and mixed and allowed to settle at 25 °C +/- 1.5 °C. Aqueous samples for analysis were removed from below the organic phase.The aqueous phase was examined for emulsions using phase contrast microscope with a magnification of 1700x and no emulsions were found.

Analysis was by gas chromatograph (GC) with a hydrogen-flame ionization detecter (Beckman). The chromatographic column was 12 ft. x 0.25 in., stainless steel tubing packed with 25% SE 30 gum rubber on 30-60 mesh firebrick. Helium flow through the column was 65cc/min.

**Test substance** : Isoprene

**Reliability** : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the test procedure and means of analysis suggest that the methodology was appropriate to evaluate the water solubility of a gaseous substance. There is otherwise no

information in the article to suggest that the data are invalid.

**Flag** : Critical study for SIDS endpoint
29.07.2005 (47)

**Solubility in** : Water
**Value** : = 642 mg/l at 25 °C
**pH value** :
**concentration** : at °C
**Temperature effects** :
**Examine different pol.** :
**PKa** : at 25 °C
**Description** :
**Stable** :
**Deg. product** :
**Method** : other: not specified
**Year** :
**GLP** : no data
**Test substance** : other TS: Isoprene

**Test substance** : Isoprene purity is unknown.
**Reliability** : (2) valid with restrictions
The value is cited in the EPIWIN experimental database (SRC Physprop Database) for isoprene. Although the original reference was not retrieved and reviewed for quality, this robust summary has a reliability rating of 2 because the data are from a peer reviewed database.
29.07.2005 (18)

**Solubility in** : Water
**Value** : = 353 mg/l at 25 °C
**pH value** :
**concentration** : at °C
**Temperature effects** :
**Examine different pol.** :
**PKa** : at 25 °C
**Description** :
**Stable** :
**Deg. product** :
**Method** : other: calculated
**Year** :
**GLP** :
**Test substance** : other TS: Isoprene

**Method** : Water solubility calculated by WSKOWWIN, a subroutine of the computer program EPIWIN version 3.11. that is based on a Kow correlation method described by W. Meylan, P. Howard and R. Boethling in "Improved method for estimating water solubility from octanol/water partition coefficient". Environ. Toxicol. Chem. 15:100-106. 1996.
**Reliability** : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured.
29.07.2005 (19)

### 2.6.2 SURFACE TENSION

## 2.7 FLASH POINT

**Value** : = -48 °C
**Type** : closed cup

**Reliability** : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.
04.04.2003 (30)

**Value** : = -54 °C
**Type** : closed cup
**Method** :
**Year** :
**GLP** : no data
**Test substance** :

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
**Reliability** : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.
27.03.2003

## 2.8 AUTO FLAMMABILITY

**Value** : = 427 °C at

**Reliability** : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.
04.04.2003 (30)

**Value** : = 220 °C at
**Method** :
**Year** :
**GLP** : no data
**Test substance** :

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
**Reliability** : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.
27.03.2003

## 2.9 FLAMMABILITY

**Result** : extremely flammable
**Method** :
**Year** :
**GLP** : no data
**Test substance** :

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
**Reliability** : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not

retrieved and reviewed for quality.

04.04.2003 (5) (30)

## 2.10 EXPLOSIVE PROPERTIES

| | | |
|---|---|---|
| **Result** | : | other: forms explosive mixtures with air |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no data |
| **Test substance** | : | |
| **Remark** | : | Explosion limits in air:<br>lower limit 1 % vol/vol<br>upper limit 9.7 % vol/vol |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |
| **Reliability** | : | (4) not assignable<br>This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality. |

27.03.2003

## 2.11 OXIDIZING PROPERTIES

| | | |
|---|---|---|
| **Result** | : | no oxidizing properties |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no data |
| **Test substance** | : | |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |
| **Reliability** | : | (4) not assignable<br>This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality. |

27.03.2003 (5)

## 2.12 DISSOCIATION CONSTANT

## 2.13 VISCOSITY

## 2.14 ADDITIONAL REMARKS

| | | |
|---|---|---|
| **Memo** | : | Isoprene is an unstable, oxidizable liquid. |
| **Remark** | : | Isoprene is highly reactive and unless inhibited undergoes explosive polymerization. Polymerization is accelerated by heat and by oxygen and even by the presence of rusty iron. Iron surfaces should be treated with a suitable reducing agent, such as sodium nitrite, before they are placed into isoprene service. When heated to decomposition, isoprene emits acrid smoke and fumes. |
| **Source** | : | Exxon Chemical Europe Inc. Bruxelles |

27.03.2003 (5)

### 3.1.1 PHOTODEGRADATION

| | | |
|---|---|---|
| **Type** | : | air |
| **Light source** | : | |
| **Light spectrum** | : | nm |
| **Relative intensity** | : | based on intensity of sunlight |
| **Conc. of substance** | : | at 25 °C |
| **INDIRECT PHOTOLYSIS** | | |
| **Sensitizer** | : | OH |
| **Conc. of sensitizer** | : | |
| **Rate constant** | : | = .00000000010514 $cm^3$/(molecule*sec) |
| **Degradation** | : | = 50 % after 1.2 hour(s) |
| **Deg. product** | : | |
| **Method** | : | other (calculated): Calculated values using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04 |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Calculated values using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04<br><br>Indirect photodegradation, or atmospheric oxidation potential, is based on the structure-activity relationship methods developed by R. Atkinson under the following conditions:<br>Temperature: 25°C<br>Sensitizer: OH- radical<br>Concentration of Sensitizer: 1.5E6 OH- radicals/cm3 |
| **Remark** | : | An approach to assessing the potential for a substance to undergo direct photochemical degradation is to assume that degradation will occur in proportion to the amount of light wavelengths >290 nm absorbed by constituent molecules (Zepp and Cline, 1977). Isoprene does not absorb light within a range of 290 to 750 nm. Therefore, isoprene is not subject to direct photolysis and this degradative mechanism will not contribute to its loss from the environment. |
| **Reliability** | : | (2) valid with restrictions<br>The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are calculated and not measured. |
| **Flag** | : | Critical study for SIDS endpoint |

29.07.2005 (18)

| | | |
|---|---|---|
| **Deg. product** | : | |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Technical discussion |
| **Remark** | : | An approach to assessing the potential for a substance to undergo direct photochemical degradation is to assume that degradation will occur in proportion to the amount of light wavelengths >290 nm absorbed by constituent molecules (Zepp and Cline, 1977). Isoprene does not absorb light within a range of 290 to 750 nm. Therefore, isoprene is not subject to direct photolysis and this degradative mechanism will not contribute to its loss from the environment. |
| **Reliability** | : | (2) valid with restrictions |
| **Flag** | : | Critical study for SIDS endpoint |

29.07.2005 (78)

| | | |
|---|---|---|
| **Type** | : | air |
| **Light source** | : | |
| **Light spectrum** | : | nm |
| **Relative intensity** | : | based on intensity of sunlight |

**Remark** : Undergoes photooxidation in the atmosphere and is decomposed to CO and CO2 (Hanst et al., 1980).

Reacts with ozone in air yielding formaldehyde (85 %), methacrolein and methylvinylketone (Niki et al., 1983).

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

**Reliability** : (4) not assignable
This robust summary has a reliability rating of 4 because the data were not retrieved and reviewed for quality.

29.07.2005 (28) (56)

### 3.1.2 STABILITY IN WATER

| | | |
|---|---|---|
| **Type** | : | abiotic |
| **t1/2 pH4** | : | at °C |
| **t1/2 pH7** | : | at °C |
| **t1/2 pH9** | : | at °C |
| **Deg. product** | : | |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no data |
| **Test substance** | : | other TS: Isoprene |

**Result** : Hydrolysis of an organic molecule can occur when a molecule (R-X) reacts with water (H2O) to form a new carbon-oxygen bond after the carbon-X bond is cleaved. Mechanistically, this reaction is referred to as a nucleophilic substitution reaction, where X is the leaving group being replaced by the incoming nucleophilic oxygen from the water molecule. The leaving group, X, must be a molecule other than carbon because for hydrolysis to occur, the R-X bond cannot be a carbon-carbon bond. This reaction differs from other reactions with water such as hydration of carbonyls that can lead to the formation of an alcohol beginning with the transfer of a proton from the water to an alkene. However, water by itself is too weak an acid to transfer a proton in the absence of a strong acid, which could effect such an acid catalysed electrophilic addition.

Thus, hydrocarbons such as alkenes are not subject to hydrolysis under conditions typically found within the environment and therefore, this fate process will not contribute to the degradative loss of 2-methyl-2-butene from the environment.

**Flag** : Critical study for SIDS endpoint

07.01.2005 (25) (29)

**Remark** : Isoprene is highly volatile and has a low water solubility. Its specific gravity is less than that of water, therefore it will float on the water (in case of a spill) and is expected to rapidly evaporate from the surface.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

31.03.2003

### 3.1.3 STABILITY IN SOIL

**Deg. product** :
**Method** :
**Year** :
**GLP** :
**Test substance** : other TS: Isoprene

**Remark** : Based on scientific judgement that considered estimated aerobic biodegradation half-life values, the biodegradation half-life of isoprene in soil is estimated to range from 7 to 28 days.
**Reliability** : (4) not assignable
This robust summary has a reliability rating of 4 because the data are an estimate based on scientific judgement.
29.07.2005 (31)

### 3.2.1 MONITORING DATA

### 3.2.2 FIELD STUDIES

### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

**Type** :
**Media** : water - air
**Air** : % (Fugacity Model Level I)
**Water** : % (Fugacity Model Level I)
**Soil** : % (Fugacity Model Level I)
**Biota** : % (Fugacity Model Level II/III)
**Soil** : % (Fugacity Model Level II/III)
**Method** : other: Henry's Law constant calculation
**Year** :

**Result** : The Henry's Law constant (HLC) representing volatility for isoprene is 7,781 Pa-m3/mole at 25°C. The HLC was calculated using a water solubility of 642 mg/L, a vapour pressure of 733.3 hPa, and a molecular weight of 68.12. The vapor pressure and water solubility values are measured values and were obtained from the Syracuse Research Corp. physprop database (EPIWIN).
**Test substance** : Isoprene
**Reliability** : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated and not measured.
**Flag** : Critical study for SIDS endpoint
29.07.2005 (18)

**Type** :
**Media** : other: air - biota - sediment(s) - soil - water
**Air** : % (Fugacity Model Level I)
**Water** : % (Fugacity Model Level I)
**Soil** : % (Fugacity Model Level I)
**Biota** : % (Fugacity Model Level II/III)
**Soil** : % (Fugacity Model Level II/III)
**Method** : other: Calculation according Mackay, Level I
**Year** :

**Remark** : Physicochemical data used in the calculation:

| Parameter | Value w/ Units |
|---|---|
| Molecular Weight | 68.12 |
| Temperature | 25° C |
| Log Kow | 2.42 |
| Water Solubility | 642 g/m3 |
| Vapor Pressure | 73330 Pa |
| Melting Point | -145.9°C |

**Result** : Using the Mackay Level I calculation, the following distribution is predicted for isoprene:

| %Distribution | Compartment |
|---|---|
| 99.92 | Air |
| 0.06 | Water |
| 0.02 | Soil |
| 0.00 | Sediment |
| 0.00 | Suspended Sediment |
| 0.00 | Biota |

**Test substance** : Isoprene
**Reliability** : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated.
**Flag** : Critical study for SIDS endpoint
29.07.2005 (42)

**Type** :
**Media** : other: air - biota - sediment(s) - soil - water
**Air** : % (Fugacity Model Level I)
**Water** : % (Fugacity Model Level I)
**Soil** : % (Fugacity Model Level I)
**Biota** : % (Fugacity Model Level II/III)
**Soil** : % (Fugacity Model Level II/III)
**Method** : other: Calculation according Mackay, Level III
**Year** :

**Remark** : Physicochemical data used in the calculation:

| Parameter | Value w/ Units |
|---|---|
| Molecular Weight | 68.12 |
| Temperature | 25° C |
| Log Kow | 2.42 |
| Water Solubility | 642 g/m3 |
| Vapor Pressure | 73330 Pa |
| Melting Point | -145.9°C |

Emissions rates used in the calculation:

| Compartment | Rate (kg/hr) |
|---|---|
| Air | 1000 |
| Water | 1000 |
| Soil | 1000 |

Half-lives used in the calculation:

| Compartment | Half-life (hr) |
|---|---|

| | | |
|---|---|---|
| Air | 1.2a |
| Water | 120b |
| Soil | 420c |
| Sediment | 420c |

a - as calculated using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04 [EPIWIN (1999). Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.]
b - based on biodegradation data from EMBSI (2004) and Boethling (2000): ExxonMobil Biomedical Sciences, Inc. (2004). Ready Biodegradability, Manometric Respirometry. Study #177294A.

Boethling R (2000). HPVC-Screening Tool: Using Ready and Inherent Biodegradability Data to Derive Input Data for the EQC Model, Appendix 10 in Environment Canada, Environmental Categorization for Persistence Bioaccumulation and Inherent Toxicity of Substances on the Domestic Substance List Using QSARs, Results of an international workshop hosted by Chemicals Evaluation Division of Environment Canada, Nov. 11-12, 1999, in Philadelphia, PA, USA.
c - based on Boethling, R. recommendation that half-lives of 3 to 4 times longer than surface water should be used for soil and sediment.

**Result** : Using the Mackay Level I calculation, the following distribution is predicted for isoprene:

| Compartment | %Distribution |
|---|---|
| Air | 3.11 |
| Water | 87.72 |
| Soil | 8.96 |
| Sediment | 0.21 |

**Test substance** : Isoprene
**Reliability** : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated.
**Flag** : Critical study for SIDS endpoint
29.07.2005 (43)

**Type** :
**Media** : other: air - biota - sediment(s) - soil - water
**Air** : % (Fugacity Model Level I)
**Water** : % (Fugacity Model Level I)
**Soil** : % (Fugacity Model Level I)
**Biota** : % (Fugacity Model Level II/III)
**Soil** : % (Fugacity Model Level II/III)
**Method** : other: Calculation according Mackay, Level III
**Year** :

**Remark** : Physicochemical data used in the calculation:

| Parameter | Value w/ Units |
|---|---|
| Molecular Weight | 68.12 |
| Temperature | 25° C |
| Log Kow | 2.42 |
| Water Solubility | 642 g/m3 |
| Vapor Pressure | 73330 Pa |
| Melting Point | -145.9°C |

Emissions rates used in the calculation:

| Compartment | Rate (kg/hr) |
|---|---|
| Air | 1000 |
| Water | 0 |
| Soil | 0 |

Half-lives used in the calculation:

| Compartment | Half-life (hr) |
|---|---|
| Air | 1.2a |
| Water | 120b |
| Soil | 420c |
| Sediment | 420c |

a - as calculated using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04 [EPIWIN (1999). Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.]
b - based on biodegradation data from EMBSI (2004) and Boethling (2000): ExxonMobil Biomedical Sciences, Inc. (2004). Ready Biodegradability, Manometric Respirometry. Study #177294A.

Boethling R (2000). HPVC-Screening Tool: Using Ready and Inherent Biodegradability Data to Derive Input Data for the EQC Model, Appendix 10 in Environment Canada, Environmental Categorization for Persistence Bioaccumulation and Inherent Toxicity of Substances on the Domestic Substance List Using QSARs, Results of an international workshop hosted by Chemicals Evaluation Division of Environment Canada, Nov. 11-12, 1999, in Philadelphia, PA, USA.
c - based on Boethling, R. recommendation that half-lives of 3 to 4 times longer than surface water should be used for soil and sediment.

**Result** : Using the Mackay Level III calculation, the following distribution is predicted for isoprene:

| Compartment | %Distribution |
|---|---|
| Air | 99.96 |
| Water | 0.02 |
| Soil | 0.02 |
| Sediment | 0.00 |

**Test substance** : Isoprene
**Reliability** : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated.
**Flag** : Critical study for SIDS endpoint
29.07.2005 (43)

**Type** :
**Media** : other: air - biota - sediment(s) - soil - water
**Air** : % (Fugacity Model Level I)
**Water** : % (Fugacity Model Level I)
**Soil** : % (Fugacity Model Level I)
**Biota** : % (Fugacity Model Level II/III)
**Soil** : % (Fugacity Model Level II/III)
**Method** : other: Calculation according Mackay, Level III
**Year** :

**Remark** : Physicochemical data used in the calculation:

| Parameter | Value w/ Units |
|---|---|

| | | |
|---|---|---|
| | | Molecular Weight 68.12<br>Temperature 25° C<br>Log Kow 2.42<br>Water Solubility 642 g/m3<br>Vapor Pressure 73330 Pa<br>Melting Point -145.9°C<br><br>Emissions rates used in the calculation:<br><br>Compartment Rate (kg/hr)<br><br>Air 0<br>Water 1000<br>Soil 0<br><br>Half-lives used in the calculation:<br><br>Compartment Half-life (hr)<br><br>Air 1.2a<br>Water 120b<br>Soil 420c<br>Sediment 420c<br><br>a - as calculated using AOPWIN version 1.89, a subroutine of the computer program EPIWIN version 3.04 [EPIWIN (1999). Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.]<br>b - based on biodegradation data from EMBSI (2004) and Boethling (2000): ExxonMobil Biomedical Sciences, Inc. (2004). Ready Biodegradability, Manometric Respirometry. Study #177294A.<br><br>Boethling R (2000). HPVC-Screening Tool: Using Ready and Inherent Biodegradability Data to Derive Input Data for the EQC Model, Appendix 10 in Environment Canada, Environmental Categorization for Persistence Bioaccumulation and Inherent Toxicity of Substances on the Domestic Substance List Using QSARs, Results of an international workshop hosted by Chemicals Evaluation Division of Environment Canada, Nov. 11-12, 1999, in Philadelphia, PA, USA.<br>c - based on Boethling, R. recommendation that half-lives of 3 to 4 times longer than surface water should be used for soil and sediment. |
| **Result** | **:** | Using the Mackay Level I calculation, the following distribution is predicted for isoprene:<br><br>Compartment %Distribution<br>Air 0.42<br>Water 99.34<br>Soil 0.00<br>Sediment 0.24 |
| **Test substance** | **:** | Isoprene |
| **Reliability** | **:** | (2) valid with restrictions<br>This robust summary has a reliability rating of 2 because the data are calculated. |
| **Flag** | **:** | Critical study for SIDS endpoint |
| 29.07.2005 | | (43) |

### 3.3.2 DISTRIBUTION

**Media** :
**Method** : other (calculation)
**Year** :

**Method** : The calculated value was determined using PCKOCWIN version 1.66, a subroutine within the computer program EPIWIN version 3.04.
**Result** : Koc = 67.7
Log Koc = 1.83
**Test substance** : Isoprene
**Reliability** : (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPIWIN. This robust summary has a reliability rating of 2 because the data are not measured.
**Flag** : Critical study for SIDS endpoint
29.07.2005 (18)

## 3.4 MODE OF DEGRADATION IN ACTUAL USE

**Memo** : Main route of isoprene degradation is through atmospheric oxidation by OH radicals. The atmospheric half-life is expected to be 1.2 hours.

**Remark** : Isoprene is expected to volatilize from soil or open waters rapidly. As isoprene volatilizes, it is subject to oxidation predominantly by hydroxyl radical attack. In the aquatic environment, isoprene is resistant to hydrolysis since it lacks hydrolyzable groups. Photolysis is an unlikely route of degradation for isoprene. Biodegradation is of minor importance since isoprene will not persist in the soil or aquatic environment long enough for a significant amount of biodegradation to occur.
**Source** : Exxon Chemical Europe Inc. Bruxelles
01.03.2004

## 3.5 BIODEGRADATION

**Type** : aerobic
**Inoculum** : activated sludge, domestic, non-adapted
**Concentration** : 51 mg/l related to Test substance
related to
**Contact time** : 28 day(s)
**Degradation** : = 61 (±) % after 28 day(s)
**Result** : readily biodegradable
**Kinetic of testsubst.** : 10 day(s) = 10 %
18 day(s) = 60 %
28 day(s) = 61 %
%
%
**Control substance** : Benzoic acid, sodium salt
**Kinetic** : 28 day(s) = 93 %
%
**Deg. product** :
**Method** : OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
**Year** : 2004
**GLP** : yes
**Test substance** : other TS: Isoprene (CAS No. 78-79-5)
**Result** : The biodegradation half-life <2 weeks. By day 28, 60.9% degradation of

the test material was observed. 10% biodegradation was achieved on approximately day 10, 50% biodegradation on approximately day 13, and >60% biodegradation on day 18. However, isoprene was not readily biodegradable because the replicate data exceeded the allowable range (53 to 75%).

By day 2, >60% biodegradation of positive control was observed, which meets the guideline requirement. No excursions from the protocol were noted.

Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test material as calculated using results of an elemental analysis of the test material.

| Sample | % Degradation* (day 28) | Mean % Degradation (day 28) |
|---|---|---|
| Isoprene | 53.4, 54.6, 74.8 | 60.9 |
| Na Benzoate | 89, 91, 100 | 93 |

* replicate data

**Test condition** : Activated sludge and test medium were combined per OECD Guideline 301F prior to test material addition. Test medium consisted of glass distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride) per OECD Guideline 301F.

Test vessels were 1L glass flasks placed in a waterbath and electronically monitored for oxygen consumption. Test material was tested in triplicate, controls and blanks were tested in duplicate.

Test material (isoprene) concentration was 51 mg/L. The positive control (sodium benzoate) concentration was 46 mg/L. Test temperature was 22 +/- 1 Deg C.

All test vessels were stirred constantly for 28 days using magnetic stir bars and plates.

**Test substance** : Isoprene (CAS No. 78-79-5)
Purity: >99%

**Conclusion** : The test material is not readily biodegradable, but exhibited a high extent of biodegradation.

**Reliability** : (2) valid with restrictions
This summary represents a key study because it followed an OECD standard guideline, which describes a procedure specifically designed to evaluate this endpoint, and the results were reviewed for reliability and assessed as valid. The study was given a reliability of 2 because the replicate data exceded the allowable range.

**Flag** : Critical study for SIDS endpoint

29.07.2005 (20)

**Type** : aerobic
**Inoculum** : other: domestic sewage effluent
**Concentration** : 2 mg/l related to Test substance
related to
**Contact time** : 28 day(s)
**Degradation** : (±) % after
**Result** :
**Deg. product** :
**Method** : other:
**Year** : 2002
**GLP** : yes
**Test substance** : other TS: Isoprene

**Method** : OECD 301D; US EPA OPPTS 835.3110; and EC Directive 92/69, C.4-E

**Result** : The mean Total Viable Count of microorganisms in the sample of final sewage effluent in the main test was 7.1 x 105 colony forming units (cfu) per ml and the mean count in inoculated mineral salts medium on day 0 was 3.3 x 104 cfu/ml.

Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test material as calculated using results of an elemental analysis of the test material.

A maximum extent of 60% biodegradation was measured in one test system on day 18 of the main Closed Bottle test. On day 28, the average biodegradation was 30% (2 and 58%). A maximum extent of 64% biodegradation was measured on day 7 of the supplementary investigation, which confirms that isoprene can be rapidly biodegraded in the presence of an acclimated inoculum. The test substance showed no inhibitory effect on the normal degradative activity of the microbial inoculum in the supplementary study.

| | % Degradation* | | |
|---|---|---|---|
| Sample | Day 5 | Day 18 | Day 28 |
| Test material | 5, 2 | 2, 60 | 2, 58 |
| Na Benzoate | 78, 79 | --- | 86, 86 |

* replicate data from non acclimated test systems

**Test condition** : A sample of secondary effluent was collected on the day of the test from a trickling-filter plant and maintained under aerobic conditions in the laboratory. Immediately before use, it was filtered through glass wool and the filtrate used as the inoculum for the test. Eighteen test system bottles were filled with a mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1 ml/L, and the test substance at a nominal loading of 2 mg/l.

Two additional series of eighteen bottles each were filled with inoculated mineral salts medium. The reference substance, sodium benzoate, at a nominal loading of 5 mg/l was added to one series, while the second series was used to determine background respiration. DO (dissolved oxygen) concentrations in replicate bottles from each series of eighteen bottles were initially measured on day 0 and following incubation at 22 ± 2°C in darkness on days 5, 7, 11, 14, 18, 21, 25, and 28. An additional four bottles, each containing inoculated mineral salts medium with test and reference substances, were used to assess the impact of the test substance on the degradative activity of the the inoculum. DO concentrations were determined on days 0 and 5.

In a supplementary investigation, eight bottles were established with mineral salts medium and an inoculum obtained from a BOD bottle in which isoprene biodegradation had achieved 58% on day 28. Four bottles contained isoprene, while the second four bottles acted as controls containing inoculated medium alone. The DO, pH, and temperature of two bottles containing isoprene and two bottles containing inoculum and mineral salts medium alone were analysed on day 0. The remaining bottles were incubated for a total of 7 days in darkness at 22 ± 2°C and then analysed.

**Test substance** : Purity: 98.6% w/w (analysis of 16/11/01)
98.5% w/w (analysis of 23/7/02)
Lot number: A0140985
Appearance: Clear, colourless liquid
Storage conditions: Cool, dry well-ventilated area in the dark under

nitrogen
Supplier: Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicester, LE11 5RG, USA
Date received by lab conducting test: 16 May 2001

The carbon content of the test substance was determined before the start of the Closed Bottle test using a CEC Model 440 Elemental Analyser. The measured carbon content (89%) was equivalent to 101% of the theoretical value (88.16%).

**Conclusion** : Isoprene has the potential to biodegrade to a significant extent under non acclimated and acclimated test conditions.

The results of selected individual replicate test systems from this study for sampling days 18 through 28 are outside guideline requirements, which brings the validity of the the study into question. However, it is equally questionable that valid replicate data would be achieved in subsequent tests under a similar test design. When the individual test system results are considered, it is clear from this study that an 18-day lag period occurred thorugh the day 14 samples (replicate day 14 results were 5 and 5%). All other test systems prior to day 14 exhibited less than 5% biodegradation. These data suggest that initiating biodegradation may be metabolically challenging. In comparison, results from day 18 through 28 replicate samples range from 54 to 60% for three samples and 2 to 13% for five samples. These data suggest that biodegradation once initiated can proceed to a high extent, but test conditions can limit the opportunity for acclimation with a subsequent high extent of biodegradation to occur.

**Reliability** : (2) valid with restrictions
This study is assigned a reliability of "2" because the range of replicate results were not within guideline requirements. No other guideline deviations occurred. Although this study was conducted according to guideline methods, the replicate test systems exhibited greater than 20% variability on days 18 through 28 of the study. However, the data from this study can still be used to evaluate the potential biodegradability of the test material with an understanding of the test system limitations and the potential for the type of results exhibited in this study.

01.03.2004 (33)

**Type** : aerobic
**Inoculum** :
**Concentration** : 2 mg/l related to Test substance
related to
**Contact time** : 28 day(s)
**Degradation** : = 2 (±) % after 28 day(s)
**Result** :
**Deg. product** :
**Method** : OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
**Year** :
**GLP** :
**Test substance** : other TS: Isoprene (CAS No. 78-79-5)

**Result** : At test concentrations of both 2 and 10 mg/L, the test material exhibited 2% biodegradation after 28 days.

**Test condition** : The test was conducted using a guideline that corresponds to the OECD 301C, Modified MITI Test. The test material was evaluated at 2 and 10 mg/L. The inoculum was developed from sludge obtained from 4 sewage plants, 3 rivers, 1 lake, and 2 bays according to methods described in the OECD guideline.

**Test substance** : Isoprene (CAS No. 78-79-5)

**Reliability** : (2) valid with restrictions

29.07.2005 (9)

| | | |
|---|---|---|
| **Type** | : | aerobic |
| **Inoculum** | : | Arthrobacter sp. (Bacteria) |
| **Concentration** | : | 385 µg/l related to<br>500 µg/l related to |
| **Method** | : | GC/PID analysis of soil core headspace. |
| **Remark** | : | Sealed oil cores were used to evaluate isoprene vapor degradation in the field and lab. Alfisol from a temperate mixed-hardwoods forest near Ithaca, NY, US had a pH 5.8-6, 9% organic matter and 57% porosity. |
| **Result** | : | Headspace isoprene concentrations fell from approx 385 ppb at time 0 to < 5ppb by 2 hr in the soil cores maintained in the field. During the four month (June - Oct 1996) experiment, headspace samples were collected monthly; after sampling the isoprene was reinjected into the core headspace. Monthly analysis for isoprene showed average degradation rates approx. 2000 pmol/gram soil/day. Soil cores maintained in the lab and dosed with isoprene @ 500 ppm showed complete removal from the headspace after 18 hours. |
| **Source** | : | Exxon Chemical Europe Inc. Bruxelles |
| **Reliability** | : | (2) valid with restrictions<br>This robust summary has a reliability rating of 2 because the data were developed using non standardized test procedures. However, the information is well documented and meets accepted scientific principles. |

29.07.2005 (10)

## 3.6 BOD5, COD OR BOD5/COD RATIO

## 3.7 BIOACCUMULATION

| | | |
|---|---|---|
| **Species** | : | other: see remark |
| **Exposure period** | : | at °C |
| **Concentration** | : | |
| **Elimination** | : | |
| **Method** | : | OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish" |
| **Year** | : | |
| **GLP** | : | no data |
| **Test substance** | : | other TS: Isoprene |
| **Remark** | : | The species tested was the carp (Cyuprinus carpio) supplied by Sugishima fish farm, Kumamoto, Japan. All fish were acclimated for approximately 28 days prior to test initiation. |
| **Result** | : | Measured log bioconcentration factor (BCF) values were determined as 0.7 to 1.1 (BCF = 5.0 to 14) and 0.7 to 1.3 (BCF = 5.6 to 20) at exposure concentrations of 50 and 5 mg/L, respectively. |
| **Test condition** | : | Test water was ground water from Kurume Research Laboratory. Water quality parameters were monitored requlary and included temperature, pH, and disolved oxygen. Additionally, total hardness, COD, and contaminants were analyzed regularly on site. Dilution water met ministerial ordinance of the Ministry of Health and Welfare for total hardness and residue.<br><br>Test temperature was 25 +/- 2 degree C. Fish weight was approximately 30g, length approximately 10 cm, and lipid content 2 to 6% body weight. Fish were fed twice daily a daily total amount corresponding to |

approximately 2% total body weight. Fish were not fed on sampling days. Water flow rate was 200 to 800 ml/min. Dissolved oxygen ranged from 6 to 8 mg/L. Up to 20 fish may have been used per study.

Samples were analyzed as follows:
Test material exposure solution - 1 sample twice a week
Test fish - 2 samples every two weeks
Control fish - 2 samples before test initiation and at termination

Recovery efficiencies were determined and used as correction factors for the determination of test substance concentration in test samples.

**Reliability** : (2) valid with restrictions
**Flag** : Critical study for SIDS endpoint
29.07.2005 (9)

**Species** : other: see remark
**Exposure period** : at °C
**Concentration** :
**BCF** : = 14.57
**Elimination** :
**Method** : other: calculation
**Year** :
**GLP** : no data
**Test substance** : other TS: Isoprene

**Remark** : A log BCF of 1.163 (BCF = 14.57) is calculated. With respect to the log Pow = 2.42, isoprene in the aquatic environment is expected to have a low potential for bioaccumulation.
**Reliability** : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated and not measured.
29.07.2005 (18)

**Remark** : Could not confirm reported log P, data not in reference, but other log P data supports low BCF potential.
The water solubility of isoprene of 0.38 g/l is such low, that bioaccumulation is not expected to occur, although the log Pow being about 3.
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
29.07.2005 (8)

## 3.8 ADDITIONAL REMARKS

**Remark** : The photochemical ozone creation potential (POCP) index for a chemical provides a relative measure of its reactivity or ozone forming potential. The POCP index can also provide a means of ranking volatile organic compounds (VOCs) by their ability to form ozone in the troposphere. Reported POCP indices for isoprene in northwestern Europe range from 109.2 to 117.8, in comparison with an POCP index of 100 for ethylene, the reference substance.
**Reliability** : (2) valid with restrictions
The values were calculated. This robust summary has a reliability rating of 2 because the data are not measured.
29.07.2005 (16) (17)

## 4.1 ACUTE/PROLONGED TOXICITY TO FISH

**Type** : semistatic
**Species** : Oncorhynchus mykiss (Fish, fresh water)
**Exposure period** : 96 hour(s)
**Unit** : mg/l
**LC50** : = 7.43 measured/nominal
**Limit test** :
**Analytical monitoring** : yes
**Method** : OECD Guide-line 203 "Fish, Acute Toxicity Test"
**Year** : 2002
**GLP** : yes
**Test substance** : other TS: Isoprene

**Result** : 96-hour LC50 = 7.43 mg/L (95% CI = 6.71 and 15.0 mg/l) based upon measured values

| Nominal Conc. (mg/L) | Mean Measured Conc. (mg/L) | Fish Total Mortality (@96 hrs)* |
|---|---|---|
| Control | Not Detected | 0 |
| 2.13 | 1.68 | 0 |
| 4.70 | 3.57 | 0 |
| 10.3 | 6.71 | 4 |
| 22.7 | 15.0 | 10 |
| 50.0 | 28.7 | 10 |

*10 fish total added to control and each treatment at test initiation

**Test condition** : Individual treatment solutions were prepared by injecting the test substance through a silicone bung into a glass vessel containing test medium with minimal headspace and stirring for approximately 24 hours to obtain equilibrium concentrations. After stirring, the solutions were left to stand for approximately 30 minutes before aliquots of the medium were removed via a sampling tube from the middle of the vessel and used to fill the test vessels.

Two replicates of each treatment and control were tested in completely filled, no headspace, aspirator bottles (capacity approximately 11.4 L) with 5 fish in each vessel. The fish were exposed for a period of 96 hours with daily batch renewal of the media. Daily renewals were performed by removing ~80% of the test solution through the port at the bottom and refilling with fresh solution.

Nominal treatment levels were: control, 2.13, 4.70, 10.3, 22.7, and 50.0 mg/L, which measured: not detected, 1.68, 3.57, 6.71, 15.0, and 28.7 mg/L, respectively. The measured values are based on the mean of test substance in samples taken from the new and old solutions.

Test temperature was 14.1 to 15.4 Deg C., Lighting was 16 hours light : 8 hours dark with periods of subdued lighting at the beginning and end of each light phase.

Dissolved Oxygen at initiation ranged from 97 to >100% of the air saturation value (ASV) and from 31 to 99% ASV in "old" solutions prior to renewals. The pH ranged from 7.3 to 8.1 during the study. Total hardness was within the range of 160 to 190 mg as CaCO3. Fish were not fed during the study.

The analytical method used was Headspace Gas Chromatography with Flame Ionization Detection (GC-FID).

**Test substance** : Identity: Isoprene
Other name: Isoprene, stabilised 99+%
Chemical name: 2-Methyl-1,3-butadiene
CAS number: 78-79-5
Lot number: A0140985
Appearance: Clear, colourless liquid
Storage conditions: Cool, dry, well ventilated area
Purity: 99.1% (supplier); 98.6% (GC assay)
Sample received: 16 May 2001
Sample source:
Fisher Scientific UK
Bishop Meadow Road
Loughborough
Leicester LE11 5RG
UK

**Reliability** : (1) valid without restriction
This summary represents a valid study because it followed an OECD standard guideline, which describes a procedure specifically designed to evaluate this endpoint, and did not include procedures or results that would support characterizing it as invalid.

**Flag** : Critical study for SIDS endpoint

03.04.2003 (34)

**Type** :
**Species** : Carassius auratus (Fish, fresh water)
**Exposure period** : 96 hour(s)
**Unit** : mg/l
**LC50** : = 180

**Remark** : LL50 values are probably lower than reported, as systems were static, and open during tests. Also reference mentions that "due to volalitility and biodegradability, and since concentrations were not measured) the eff. concentrations became lower with time, but only nominal cons were used. Test systems were static, and open, which allowed for volatile loss of test substance.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

**Reliability** : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.

29.07.2005 (60)

**Type** :
**Species** : Lepomis macrochirus (Fish, fresh water)
**Exposure period** : 96 hour(s)
**Unit** : mg/l
**LC50** : = 43

**Remark** : LL50 values are probably lower than reported, as systems were static, and open during tests. Also reference mentions that "due to volalitility and biodegradability, and since concentrations were not measured) the eff. concentrations became lower with time, but only nominal cons were used. Test systems were static, and open, which allowed for volatile loss of test substance.

**Source** : Deutsche Shell Chemie GmbH Eschborn

Exxon Chemical Europe Inc. Bruxelles

**Reliability** : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.

29.07.2005 (60)

**Type** :
**Species** : Pimephales promelas (Fish, fresh water)
**Exposure period** : 96 hour(s)
**Unit** : mg/l
**LC50** : = 75

**Remark** : Hard water
LL50 values are probably lower than reported, as systems were static, and open during tests. Also reference mentions that "due to volalitility and biodegradability, and since concentrations were not measured) the eff. concentrations became lower with time, but only nominal cons were used.
Test systems were static, and open, which allowed for volatile loss of test substance.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

**Reliability** : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.

29.07.2005 (60)

**Type** :
**Species** : Pimephales promelas (Fish, fresh water)
**Exposure period** : 96
**Unit** : mg/l
**LC50** : = 87

**Remark** : LL50 values are probably lower than reported, as systems were static, and open during tests. Also reference mentions that "due to volalitility and biodegradability, and since concentrations were not measured) the eff. concentrations became lower with time, but only nominal cons were used.
Soft water
Test systems were static, and open, which allowed for volatile loss of test substance.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

**Reliability** : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.

29.07.2005 (60)

## 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

**Type** : Static
**Species** : Daphnia magna (Crustacea)
**Exposure period** : 48 hour(s)
**Unit** : mg/l
**EC50** : = 5.77 measured/nominal

**Analytical monitoring** : Yes
**Method** : OECD Guide-line 202
**Year** : 2002
**GLP** : Yes
**Test substance** : other TS: Isoprene

**Result** : 48-hour EC50 = 5.77 mg/L (95% CI = 3.52 and 9.47 mg/l) based upon measured values

| Nominal Conc. (mg/L) | Mean Measured Conc. (mg/L) | Daphnid Immobility (@48 hrs)* |
|---|---|---|
| Control | Not Detected | 0 |
| 2.13 | 0.65 | 0 |
| 4.70 | 1.55 | 0 |
| 10.3 | 3.52 | 0 |
| 22.7 | 9.47 | 20 |
| 50.0 | 24.6 | 20 |

*20 daphnids total added to control and each treatment at test initiation

**Test condition** : Individual treatment solutions were prepared by injecting the test substance through a silicone bung into a glass vessel containing test medium with minimal headspace and stirring for approximately 22 hours to obtain equilibrium concentrations. After stirring, the solutions were left to stand for approximately 30 minutes before aliquots of the medium were removed via a sampling tube from the middle of the vessel and used to fill the test vessels.

Two replicates of each treatment and control were tested in completely filled, no headspace, glass vessels with 10 daphnids in each vessel. The daphnids were exposed for a period of 48 hours without renewal of the media.

Nominal treatment levels were: control, 2.13, 4.70, 10.3, 22.7, and 50.0 mg/L, which measured: not detected, 0.648, 1.55, 3.52, 9.47, and 24.6 mg/L, respectively. The measured values are based on the mean of test substance in samples taken from the new and old (test termination) solutions.

Test temperature was 14.1 to 15.4 Deg C., Lighting was 16 hours light : 8 hours dark with periods of subdued lighting at the beginning and end of each light phase.

Dissolved Oxygen at initiation ranged from 97 to >100% of the air saturation value (ASV) and from 31 to 99% ASV in "old" solutions prior to renewals. The pH ranged from 7.3 to 8.1 during the study. Total hardness was within the range of 160 to 190 mg as CaCO3. Daphnids were not fed during the study.

The analytical method used was Headspace Gas Chromatography with Flame Ionization Detection (GC-FID).

**Test substance** : Identity: Isoprene
Other name: Isoprene, stabilised 99+%
Chemical name: 2-Methyl-1,3-butadiene
CAS number: 78-79-5
Lot number: A0140985
Appearance: Clear, colourless liquid
Storage conditions: Cool, dry, well ventilated area
Purity: 99.1% (supplier); 98.6% (GC assay)
Sample received: 16 May 2001

Sample source:
Fisher Scientific UK
Bishop Meadow Road
Loughborough
Leicester LE11 5RG
UK

**Reliability** : (1) valid without restriction
This summary represents a valid study because it followed an OECD standard guideline, which describes a procedure specifically designed to evaluate this endpoint, and did not include procedures or results that would support characterizing it as invalid.
**Flag** : Critical study for SIDS endpoint
07.01.2005 (35)

**Type** :
**Species** : Daphnia magna (Crustacea)
**Exposure period** : 24 hour(s)
**Unit** : mg/l
**EC50** : = 260
**Method** :
**Year** :
**GLP** : no data
**Test substance** :

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
**Reliability** : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.
29.07.2005 (66)

**Type** :
**Species** : Daphnia magna (Crustacea)
**Exposure period** : 48 hour(s)
**Unit** : mg/l
**EC50** : = 140
**Method** :
**Year** :
**GLP** : no data
**Test substance** :

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
**Reliability** : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.
29.07.2005 (66)

## 4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

**Species** : other algae: Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) (Algae)
**Endpoint** : Biomass
**Exposure period** : 96 hour(s)
**Unit** : mg/l
**NOEC** : = 1.68 measured/nominal

**EC50** : = 15.5 measured/nominal
**Limit test** :
**Analytical monitoring** : Yes
**Method** : OECD Guide-line 201 "Algae, Growth Inhibition Test"
**Year** : 2002
**GLP** : Yes
**Test substance** : other TS: Isoprene

**Result** : Area under the growth curve:
EbC50 (72 h): 15.3 mg/l (95% CI = 12.9 and 18.6 mg/l)
EbC50 (96 h): 15.5 mg/l (95% CI = 13.3 and 18.4 mg/l)
No observed effect concentration (NOEC, 72/96 h): 1.68 mg/l

Average specific growth rate:
ErC50 (72h): >35.2 mg/l (34% inhibition at 35.2 mg/L)
ErC50 (96 h): >35.2 mg/l (31% inhibition at 35.2 mg/L)
No observed effect concentration (NOEC, 72/96 h): 6.00 mg/l

| Nominal Conc. (mg/L) | Mean Measured Conc. (mg/L) | Alga Cell Count (cells/ml @96 hrs) |
|---|---|---|
| Control | Not Detected | 117 E |
| 4.27 | 0.85 | 117 E |
| 9.39 | 1.68 | 110 E |
| 20.7 | 6.00 | 111 E |
| 45.5 | 10.3 | 74.9 E |
| 100.0 | 35.2 | 28.1 E |

**Test condition** : Treatment solutions were prepared either individually or by dilution of a treatment solution. Individual treatment solutions were prepared by injecting the test substance through a silicone bung into a glass vessel containing test medium with minimal headspace and stirring for approximately 21 hours to obtain equilibrium concentrations. After stirring, the solutions were left to stand for approximately 30 minutes before aliquots of the medium were removed via a sampling tube from the middle of the vessel and used to fill the test vessels.

Two replicates of each treatment and control were tested in completely filled, no headspace, glass vessels with an initial cell density of 1 x 104/ml. The algae were exposed for a period of 96 hours without renewal of the media.

Nominal treatment levels were: control, 4.27, 9.39, 20.7, 45.5, and 100 mg/L, which measured: not detected, 0.846, 1.68, 6.00, 10.3, and 35.2 mg/L, respectively. The measured values are based on the mean of test substance in samples taken from the new and old (test termination) solutions.

The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 23.4 to 24.0 Deg C. The pH ranged from 7.3 to 8.1 during the study.

The analytical method used was Headspace Gas Chromatography with Flame Ionization Detection (GC-FID).

**Test substance** : Identity: Isoprene
Other name: Isoprene, stabilised 99+%
Chemical name: 2-Methyl-1,3-butadiene
CAS number: 78-79-5
Lot number: A0140985
Appearance: Clear, colourless liquid
Storage conditions: Cool, dry, well ventilated area

Purity: 99.1% (supplier); 98.6% (GC assay)
Sample received: 16 May 2001
Sample source:
Fisher Scientific UK
Bishop Meadow Road
Loughborough
Leicester LE11 5RG
UK

**Reliability** : (1) valid without restriction
This summary represents a valid study because it followed an OECD standard guideline, which describes a procedure specifically designed to evaluate this endpoint, and did not include procedures or results that would support characterizing it as invalid.

**Flag** : Critical study for SIDS endpoint

29.07.2005 (36)

**Species** : other algae: green alga
**Endpoint** :
**Exposure period** : 96 hour(s)
**Unit** : mg/l
**ChV*** : = 1.83 calculated
**Method** :
**Year** :
**GLP** :
**Test substance** : other TS: Isoprene

**Test condition** : A log Kow (octanol/water partition coefficient) value and a chemical structure are needed to complete the calculation with this model. The log Kow value used was 2.42 (Hansch and Leo). The SMILES (Simplified Molecular Input Line Entry System) structure used with the model was: C=C(C)C=C. Additional data calculated by the model include molecular weight, 68.12, and water solubility, 112.9 mg/L (@25C).

**Reliability** : (2) valid with restrictions
This robust summary has a reliability rating of 2 because the data are calculated and not measured.

01.03.2004 (18) (27)

**Species** : Scenedesmus quadricauda (Algae)
**Endpoint** : growth rate
**Exposure period** : 96 hour(s)
**Unit** : mg/l
**EC50** : > 1000
**Method** :
**Year** :
**GLP** : no data
**Test substance** :

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

**Reliability** : (3) invalid
This study is rated a 3 for reliability because it is unlikely that the test procedure maintained exposure concentrations based on results of studies using current testing techniques.

29.07.2005 (67)

## 4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

### 4.5.1 CHRONIC TOXICITY TO FISH

| | | |
|---|---|---|
| **Species** | : | other: fish |
| **Endpoint** | : | |
| **Exposure period** | : | 30 day(s) |
| **Unit** | : | mg/l |
| **ChV*** | : | = 2.81 calculated |
| **Method** | : | other: ECOSAR Computer Model (in:EPIWIN) |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | other TS: Isoprene |
| **Remark** | : | Test Type: Chronic Fish Toxicty Calculation |
| **Test condition** | : | A log Kow (octanol/water partition coefficient) value and a chemical structure are needed to complete the calculation with this model. The log Kow value used was 2.42 (Hansch and Leo). The SMILES (Simplified Molecular Input Line Entry System) structure used with the model was: C=C(C)C=C. Additional data calculated by the model include molecular weight, 68.12, and water solubility, 112.9 mg/L (@25C). |
| **Reliability** | : | (2) valid with restrictions<br>This robust summary has a reliability rating of 2 because the data are calculated and not measured. |

31.03.2003 (18) (27)

### 4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

| | | |
|---|---|---|
| **Species** | : | other: Daphnid |
| **Endpoint** | : | |
| **Exposure period** | : | 16 day(s) |
| **Unit** | : | mg/l |
| **EC50** | : | = 1.38 calculated |
| **Method** | : | other: ECOSAR Computer Model (in:EPIWIN) |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | other TS: Isoprene |
| **Remark** | : | Test Type: Chronic Daphnid Toxicity Calculation |
| **Test condition** | : | A log Kow (octanol/water partition coefficient) value and a chemical structure are needed to complete the calculation with this model. The log Kow value used was 2.42 (Hansch and Leo). The SMILES (Simplified Molecular Input Line Entry System) structure used with the model was: C=C(-C)-C=C. Additional data calculated by the model include molecular weight, 68.12, and water solubility, 112.9 mg/L (@25C). |
| **Reliability** | : | (2) valid with restrictions<br>This robust summary has a reliability rating of 2 because the data are calculated and not measured. |

31.03.2003 (18) (27)

### 4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

### 4.6.2 TOXICITY TO TERRESTRIAL PLANTS

### 4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

| | | |
|---|---|---|
| **Type** | : | other: Earthworm Toxicity Calculation |
| **Species** | : | other: earthworm |
| **Endpoint** | : | Mortality |
| **Exposure period** | : | 14 day(s) |
| **Unit** | : | other: ppm |
| **LC50** | : | = 311.11 calculated |
| **Method** | : | other: ECOSAR Computer Model (in: EPIWIN) |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | other TS: Isoprene |
| **Test condition** | : | A log Kow (octanol/water partition coefficient) value and a chemical structure are needed to complete the calculation with this model. The log Kow value used was 2.42 (Hansch and Leo). The SMILES (Simplified Molecular Input Line Entry System) structure used with the model was: C=C(-C)-C=C. Additional data calculated by the model include molecular weight, 68.12, and water solubility, 112.9 mg/L (@25C). |
| **Reliability** | : | (2) valid with restrictions<br>This robust summary has a reliability rating of 2 because the data are calculated and not measured. |

31.03.2003 (18) (27)

### 4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

### 4.7 BIOLOGICAL EFFECTS MONITORING

### 4.8 BIOTRANSFORMATION AND KINETICS

### 4.9 ADDITIONAL REMARKS

## 5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

### 5.1.1 ACUTE ORAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | = 2043 - 2210 mg/kg bw |
| **Species** | : | rat |
| **Strain** | : | Wistar |
| **Sex** | : | male/female |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **Doses** | : | |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no data |
| **Test substance** | : | other TS: isoprene in oil |
| **Remark** | : | 15 male/15 female Wistar rats were administered single doses of isoprene in oil by stomach tube. LD50 was calculated. |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |
| **Reliability** | : | (4) not assignable<br>Insufficient experimental detail to assess quality. |

29.07.2005 (38)

### 5.1.2 ACUTE INHALATION TOXICITY

| | | |
|---|---|---|
| **Type** | : | LC50 |
| **Value** | : | = 180 mg/l |
| **Species** | : | rat |
| **Strain** | : | |
| **Sex** | : | no data |
| **Number of animals** | : | |
| **Vehicle** | : | other: Not applicable |
| **Doses** | : | |
| **Exposure time** | : | 4 hour(s) |
| **Method** | : | other |
| **Year** | : | 1969 |
| **GLP** | : | |
| **Test substance** | : | other TS: Isoprene |
| **Remark** | : | No clinical observations or necropsy findings reported. Objective of study was to determine hydrocarbon concentrations in various tissues at lethal exposure concentrations. |
| **Result** | : | Rat LC50 (4 hr) = 180 mg/L (64,620 ppm); confidence limits 130-181 mg/L ($p<0.05$).<br>Mouse LC50 (2 hr) = 157 mg/L (56,363 ppm); confidence limits 129-252 mg/L ($p<0.05$). |
| **Test condition** | : | Age, number, and sex of test animals not specified. Number of groups and exposure concentrations not specified. Dynamic flow exposure system; no description of exposure chambers or conditions. Rats exposed four hours; mice exposed two hours. No post-exposure observation period - mortality study only. Exposure concentrations "controlled" by gas chromatography. LC50 calculation by probit-analysis according to Litchfield and Wilcoxon. |
| **Conclusion** | : | LC50 value reported to be 180 mg/L (64,620 ppm) in rats, 157 mg/L |

(56,363 ppm) in mice.

**Reliability** : (4) not assignable
Lethality study only; insufficient experimental detail to assess quality.

29.07.2005 (68)

**Type** : LC50
**Value** : = 157 mg/l
**Species** : mouse
**Strain** :
**Sex** : no data
**Number of animals** :
**Vehicle** : other: Not applicable
**Doses** :
**Exposure time** : 2 hour(s)
**Method** : other
**Year** : 1969
**GLP** :
**Test substance** : other TS: Isoprene

**Remark** : No clinical observations or necropsy findings reported. Objective of study was to determine hydrocarbon concentrations in various tissues at lethal exposure concentrations.

**Result** : Rat LC50 (4 hr) = 180 mg/L (64,620 ppm); confidence limits 130-181 mg/L ($p<0.05$).
Mouse LC50 (2 hr) = 157 mg/L (56,363 ppm); confidence limits 129-252 mg/L ($p<0.05$).

**Test condition** : Age, number, and sex of test animals not specified. Number of groups and exposure concentrations not specified. Dynamic flow exposure system; no description of exposure chambers or conditions. Rats exposed four hours; mice exposed two hours. No post-exposure observation period - mortality study only. Exposure concentrations "controlled" by gas chromatography. LC50 calculation by probit-analysis according to Litchfield and Wilcoxon.

**Conclusion** : LC50 value reported to be 180 mg/L (64,620 ppm) in rats, 157 mg/L (56,363 ppm) in mice.

**Reliability** : (4) not assignable
Lethality study only; insufficient experimental detail to assess quality.

29.07.2005 (69)

**Type** : LC50
**Value** : = 157 mg/l
**Species** : mouse
**Strain** :
**Sex** :
**Number of animals** :
**Vehicle** :
**Doses** :
**Exposure time** : 2 hour(s)
**Method** :
**Year** :
**GLP** : no
**Test substance** :

**Remark** : No data on number and sex of animals and on post observation period.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

**Reliability** : (4) not assignable
Lethality study only; insufficient experimental detail to assess quality.

29.07.2005 (44)

| | | |
|---|---|---|
| **Type** | : | LC50 |
| **Value** | : | = 214 mg/l |
| **Species** | : | mouse |
| **Strain** | : | |
| **Sex** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **Doses** | : | |
| **Exposure time** | : | 4 hour(s) |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no |
| **Test substance** | : | |
| **Remark** | : | No data on number and sex of animals and on post observation period. |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |
| **Reliability** | : | (4) not assignable<br>Lethality study only; insufficient experimental detail to assess quality. |

29.07.2005 (44)

| | | |
|---|---|---|
| **Type** | : | LC50 |
| **Value** | : | = 135 - 153 mg/l |
| **Species** | : | mouse |
| **Strain** | : | |
| **Sex** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **Doses** | : | |
| **Exposure time** | : | 2 hour(s) |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no |
| **Test substance** | : | |
| **Remark** | : | males: LC50 = 135-143 mg/l<br>females: LC50 = 144-153 mg/l<br>No data on number of animals and post observation period. |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |
| **Reliability** | : | (4) not assignable<br>Lethality study only; insufficient experimental detail to assess quality. |

29.07.2005 (24)

### 5.1.3 ACUTE DERMAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | |
| **Species** | : | rat |
| **Strain** | : | |
| **Sex** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **Doses** | : | |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no |

**Test substance** :

**Remark** : 5 rats received single application of 1 ml isoprene each on their back skin (animals had been shaved the day before application). The substance was not removed for 7 days. An LD50 of > 1 ml/kg b. w. was established.
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

04.04.2003 (38)

### 5.1.4 ACUTE TOXICITY, OTHER ROUTES

**Type** : LD50
**Value** : = 1310 - 1470 mg/kg bw
**Species** : rat
**Strain** : Wistar
**Sex** : male
**Number of animals** :
**Vehicle** :
**Doses** :
**Route of admin.** : i.p.
**Exposure time** :
**Method** :
**Year** :
**GLP** : no data
**Test substance** :

**Remark** : Doses from 100 to 1750 mg/kg b. w. were administered.
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

04.04.2003 (38)

### 5.2.1 SKIN IRRITATION

**Species** : rabbit
**Concentration** :
**Exposure** :
**Exposure time** :
**Number of animals** :
**Vehicle** :
**PDII** :
**Result** : slightly irritating
**Classification** : not irritating
**Method** :
**Year** :
**GLP** : no data
**Test substance** : other TS: distillate cut containing 50 % isoprene and 50 % C5

**Remark** : Prolonged contact, slight redness;
repeated contact, slight chemical burn.
Not absorbed in toxic amounts in a 3-day occlusion test.
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
**Reliability** : (4) not assignable
Insufficient experimental detail to assess quality.

29.07.2005 (37)

| | | |
|---|---|---|
| **Species** | : | rabbit |
| **Concentration** | : | |
| **Exposure** | : | |
| **Exposure time** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **PDII** | : | |
| **Result** | : | slightly irritating |
| **Classification** | : | not irritating |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no data |
| **Test substance** | : | |
| **Remark** | : | One ear each of 2 New Zealand rabbits were painted on 5 consecutive days 2 times a day with isoprene. A reversible redness staying for a short while resulted. |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |
| **Reliability** | : | (4) not assignable<br>Insufficient experimental detail to assess quality. |

29.07.2005 (38)

| | | |
|---|---|---|
| **Species** | : | rat |
| **Concentration** | : | |
| **Exposure** | : | |
| **Exposure time** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **PDII** | : | |
| **Result** | : | slightly irritating |
| **Classification** | : | not irritating |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no |
| **Test substance** | : | |
| **Remark** | : | Isoprene penetrates the intact skin and causes local irritations. |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |
| **Reliability** | : | (4) not assignable<br>Insufficient experimental detail to assess quality. |

29.07.2005 (24)

| | | |
|---|---|---|
| **Species** | : | mouse |
| **Concentration** | : | |
| **Exposure** | : | |
| **Exposure time** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **PDII** | : | |
| **Result** | : | slightly irritating |
| **Classification** | : | not irritating |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no |
| **Test substance** | : | |
| **Remark** | : | Isoprene penetrates the intact skin and causes local |

irritations.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

**Reliability** : (4) not assignable
Insufficient experimental detail to assess quality.

29.07.2005 (24)

**Species** : rabbit
**Concentration** :
**Exposure** :
**Exposure time** :
**Number of animals** :
**Vehicle** :
**PDII** :
**Result** : slightly irritating
**Classification** : not irritating
**Method** :
**Year** :
**GLP** : no
**Test substance** :

**Remark** : Isoprene penetrates the intact skin and causes local irritations.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

**Reliability** : (4) not assignable
Insufficient experimental detail to assess quality.

29.07.2005 (24)

### 5.2.2 EYE IRRITATION

**Remark** : Isoprene caused eye irritation. No further data given.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

**Reliability** : (4) not assignable
Insufficient experimental detail to assess quality.

29.07.2005 (44)

### 5.3 SENSITIZATION

### 5.4 REPEATED DOSE TOXICITY

**Type** :
**Species** : rat
**Sex** : male/female
**Strain** : Fischer 344
**Route of admin.** : inhalation
**Exposure period** : 6 hours/day
**Frequency of treatm.** : 5 days/week for 2 weeks
**Post exposure period** : not applicable
**Doses** : 0, 438, 875, 1750, 3500, or 7000 ppm
**Control group** : yes
**NOAEL** : = 7000 ppm
**LOAEL** : > 7000 ppm

| | | |
|---|---|---|
| **Method** | : | other |
| **Year** | : | 1990 |
| **GLP** | : | yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Group mean body weights, organ weights, organ weight ratios, and clinical pathology results compared to controls by Dunnett's t-test. |
| **Remark** | : | Control group and treatment: air-only exposed |
| **Result** | : | In rats, there were no exposure-related effects observed for survival, body weight gain, clinical signs, hematologic or clinical chemistry parameters, organ weights, or the incidence of gross or microscopic lesions. |
| **Test condition** | : | Groups of 20 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for two weeks (10 exposures). Ten animals/sex/group were used for clinical pathology evaluations after 4 exposures. The remaining ten animals per group were used for histopathology at the end of the study. Body weights and clinical observations were recorded weekly. Necropsies were performed and major tissues/organs preserved. Histopathologic examinations were performed on the control and high exposure animals (7000 ppm), and lower dose groups until an apparent no-observed -effect level was found. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 99%. |
| **Conclusion** | : | Isoprene exposures over 2 weeks produced no changes in any of the measured parameters in the rat at exposures up to 7000 ppm. |
| **Reliability** | : | (1) valid without restriction<br>Comparable to guideline study (OECD 412). |

29.07.2005 (48)

| | | |
|---|---|---|
| **Type** | : | |
| **Species** | : | mouse |
| **Sex** | : | male/female |
| **Strain** | : | B6C3F1 |
| **Route of admin.** | : | inhalation |
| **Exposure period** | : | 6 hours/day |
| **Frequency of treatm.** | : | 5 days/week for 2 weeks |
| **Post exposure period** | : | not applicable |
| **Doses** | : | 0, 438, 875, 1750, 3500, or 7000 ppm |
| **Control group** | : | yes |
| **LOAEL** | : | = 438 ppm |
| **Method** | : | other |
| **Year** | : | 1990 |
| **GLP** | : | yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Group mean body weights, organ weights, organ weight ratios, and clinical pathology results compared to controls by Dunnett's t-test. |
| **Remark** | : | Control group and treatment: air-only exposed |
| **Result** | : | In mice, there were no effects on survival; the mean body weight gain of males in the 7,000 ppm group was less than that of the controls. In mice, exposure to isoprene caused decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts in all exposed groups. Organ weight changes were observed in both male and female mice; increased liver weights and decreased thymus, spleen, and testis weights were observed in all exposed groups. Microscopic lesions observed in the exposed mice included atrophy of the testis and thymus, cytoplasmic vacuolization of the liver, olfactory epithelial degeneration in the nasal cavity, and epithelial hyperplasia in the forestomach. |
| **Test condition** | : | Groups of 20 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for two weeks (10 exposures). Ten animals/sex/group were used for clinical |

pathology evaluations after 5 exposures. The remaining ten animals per group were used for histopathology at the end of the study. Body weights and clinical observations were recorded weekly. Necropsies were performed and major tissues/organs preserved. Histopathologic examinations were performed on the control and high exposure animals (7000 ppm), and lower dose groups until an apparent no-observed -effect level was found.

**Test substance** : Isoprene (CAS# 78-79-5) purity > 99%.
**Conclusion** : Isoprene exposures over 2 weeks induced changes in hematological parameters, body and organ weights, and microscopic appearances in certain tissues at levels as low as 438 ppm.
**Reliability** : (1) valid without restriction
Comparable to guideline study (OECD 412).

29.07.2005 (48)

**Type** :
**Species** : Rat
**Sex** : male/female
**Strain** : Fischer 344
**Route of admin.** : Inhalation
**Exposure period** : 6 hours/day
**Frequency of treatm.** : 5 days/week for 13 weeks
**Post exposure period** : not applicable
**Doses** : 0, 70, 220, 700, 2200, or 7000 ppm
**Control group** : Yes
**NOAEL** : = 7000 ppm
**LOAEL** : > 7000 ppm
**Method** : other
**Year** : 1994
**GLP** : Yes
**Test substance** : other TS: Isoprene

**Method** : Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods.
**Remark** : Control group and treatment: air-only exposed
**Result** : In rats, there were no exposure-related effects observed for survival, body weight gain, clinical signs of toxicity, hematology or clinical chemistry parameters, urinalysis, organ weights, or the incidence of gross or microscopic lesions.
**Test condition** : Groups of 10 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Body weights and clinical observations were recorded weekly. Blood samples were collected for clinical pathology evaluations on days 4, 24, and at the end of the study. Urine samples were collected from rats during week 12. After thirteen weeks of exposures, all rats were sacrificed and evaluated histopathologically. Organ weights were recorded.
**Test substance** : Isoprene (CAS# 78-79-5) purity > 99%.
**Conclusion** : No toxicological effects were evident in rats exposed up to 7000 ppm isoprene for 13 weeks.
**Reliability** : (1) valid without restriction
Comparable to guideline study (OECD 413).

29.07.2005 (49)

**Type** :
**Species** : Mouse
**Sex** : male/female
**Strain** : B6C3F1
**Route of admin.** : Inhalation

| | | |
|---|---|---|
| **Exposure period** | : | 6 hours/day |
| **Frequency of treatm.** | : | 5 days/week for 13 weeks |
| **Post exposure period** | : | not applicable |
| **Doses** | : | 0, 70, 220, 700, 2200, or 7000 ppm |
| **Control group** | : | Yes |
| **NOAEL** | : | = 220 ppm |
| **LOAEL** | : | = 700 ppm |
| **Method** | : | other |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods. |
| **Remark** | : | Control group and treatment: air-only exposed |
| **Result** | : | In mice, there were no effects on survival, body weight gain, or clinical signs. However, male and female mice exposed to 700 ppm and higher showed hematologic effects indicative of a nonresponsive, macrocytic anemia at day 24 and after thirteen weeks. The incidences of focal epithelial hyperplasia of the forestomach were 0, 0, 0, 9, 8, 9 in the males, and 0, 0, 0, 10, 9, 10 in the females at 0, 70, 220, 700, 2200, and 7000 ppm (n=10). Degeneration of the olfactory epithelium and cytoplasmic degeneration of the liver were observed in 10/10 male mice at 7000 ppm. The male mice exposed to 7000 ppm exhibited testicular weights reduced 35% compared to the controls. |
| **Test condition** | : | Groups of 10 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Body weights and clinical observations were recorded weekly. Blood samples were collected for clinical pathology evaluations on days 4, 24, and at the end of the study. Urine samples were collected from mice during week 12. After thirteen weeks of exposures, all mice were sacrificed and evaluated histopathologically. Organ weights were recorded. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 99%. |
| **Conclusion** | : | In mice, hematological and histopathological changes were observed at exposures of 700 ppm and higher. This 13-week subchronic inhalation study, conducted as part of a 26-week carcinogenicity study, confirmed the species difference between rats and mice in susceptibility to isoprene. |
| **Reliability** | : | (1) valid without restriction<br>Comparable to guideline study (OECD 413). |

29.07.2005 (49)

| | | |
|---|---|---|
| **Type** | : | |
| **Species** | : | Rat |
| **Sex** | : | Male |
| **Strain** | : | Fischer 344 |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 6 hours/day |
| **Frequency of treatm.** | : | 5 days/week for 26 weeks |
| **Post exposure period** | : | 26-week post-exposure recovery period |
| **Doses** | : | 0, 70, 220, 700, 2200, or 7000 ppm |
| **Control group** | : | Yes |
| **NOAEL** | : | 2200 ppm |
| **LOAEL** | : | = 7000 ppm |
| **Method** | : | other |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |

| | | |
|---|---|---|
| **Method** | : | Analysis of survival and incidence of nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods. |
| **Remark** | : | Control group and treatment: air-only exposed |
| **Result** | : | The only effect observed in the male rats after 26 weeks of exposure was interstitial cell hyperplasia of the testis (10/10) in the 7000 ppm group; following the 26-week recovery period the only effect in rats was a marginal increase in benign testicular interstitial cell tumors (9/30 at 7000 ppm). |
| **Test condition** | : | Groups of 40 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 26 weeks. At the end of the 26-week exposure period, 10 rats/group were sacrificed and evaluated. The remaining animals were allowed to recovery for an additional 26 weeks without exposure at which time they were also sacrificed and evaluated. Body weights and clinical observations were recorded weekly throughout the study. Blood samples were collected for clinical pathology evaluations after 26 weeks exposure. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically. Organ weights were recorded at both intervals. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 99%. |
| **Conclusion** | : | The only effect observed in the male rats after 26 weeks of exposure was interstitial cell hyperplasia of the testis in the 7000 ppm group. Following the 26 week recovery period a marginal increase in benign testicular interstitial cell tumors was observed in the highest dose group, i.e. 7000 ppm. |
| **Reliability** | : | (2) valid with restrictions<br>Comparable to guideline studies. This study involved exposures of male rats to isoprene for 6 months, therefore provided additional data on repeated dose toxicity. |

29.07.2005 (49)

| | | |
|---|---|---|
| **Type** | : | |
| **Species** | : | Mouse |
| **Sex** | : | Male |
| **Strain** | : | B6C3F1 |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 6 hours/day |
| **Frequency of treatm.** | : | 5 days/week for 26 weeks |
| **Post exposure period** | : | 26-week post-exposure recovery period |
| **Doses** | : | 0, 70, 220, 700, 2200, or 7000 ppm |
| **Control group** | : | Yes |
| **NOAEL** | : | = 70 ppm |
| **LOAEL** | : | = 700 ppm |
| **Method** | : | other |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |

| | | |
|---|---|---|
| **Method** | : | Analysis of survival and incidence of nonneoplastic lesions was performed. Clinical chemistry, hematology, and urine data were analyzed by nonparametric methods. |
| **Remark** | : | Control group and treatment: air-only exposed |
| **Result** | : | Survival of mice was reduced in the 7000 ppm group; early deaths were attributed to various neoplastic lesions and moribund sacrifices due to hindlimb paralysis. Non-neoplastic lesions were observed in male mice exposed to isoprene and included spinal cord degeneration (>70 ppm) and degeneration of the olfactory epithelium (>220 ppm). Slight increases in testicular atrophy, epithelial hyperplasia of the forestomach, partial hindlimb paralysis and a nonresponsive macrocytic anemia were also seen in male mice. |

Selected non-neoplastic lesions were as follows (0, 70, 220, 700, 2200, 7000 ppm) -
After 26 weeks exposure:
Nasal turbinates/olfactory epithelial degeneration - 0/10, 0/10, 0/10, 1/10, 1/10, 10/10.
Testes/atrophy - 0/10, 0/10, 0/10, 0/10, 1/10, 5/10.
Spinal cord/degeneration - 0/10, 0/10, 0/10, 0/10, 1/10, 10/10.
After 26 weeks recovery:
Nasal turbinates/olfactory epithelial degeneration - 1/30, 2/30, 5/29, 11/30, 25/30, 28/28.
Testes/atrophy - 0/30, 0/30, 0/29, 0/30, 0/30, 3/29.
Spinal cord/degeneration - 4/30, 20/30, 19/29, 17/29, 13/28.

**Test condition** : Groups of 40 animals/sex/group (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 26 weeks. At the end of the 26-week exposure period, 10 mice/group were sacrificed and evaluated. The remaining animals were allowed to recovery for an additional 26 weeks without exposure at which time they were also sacrificed and evaluated. Body weights and clinical observations were recorded weekly throughout the study. Blood samples were collected for clinical pathology evaluations after 26 weeks exposure. Tissues preserved at the 26 and 52 week sacrifices were examined microscopically. Organ weights were recorded at both intervals. Twenty mice/group were evaluated for forelimb and hindlimb grip strength after 26 weeks exposure; 10 mice/group were also evaluated at 2 days, 1-, 3-, and 6-months post-exposure.

**Test substance** : Isoprene (CAS# 78-79-5) purity > 99%.

**Conclusion** : Non-neoplastic lesions related to treatment included forestomach squamous-cell hyperplasia, lung alveolar hyperplasia, nasal olefactory degeneration, and spinal cord degeneration.

**Reliability** : (2) valid with restrictions
Comparable to guideline studies. This study involved exposures of male rats and male mice to isoprene for 6 months, therefore provided additional data on repeated dose toxicity and carcinogenicity.

29.07.2005 (49)

**Type** :
**Species** : Rat
**Sex** : male/female
**Strain** : Fischer 344
**Route of admin.** : Inhalation
**Exposure period** : 6 hours/day
**Frequency of treatm.** : 5 days/week for 104 weeks
**Post exposure period** : None
**Doses** : 0, 220, 700, or 7000 ppm
**Control group** : Yes
**Method** : other
**Year** : 1997
**GLP** : Yes
**Test substance** : other TS: Isoprene

**Method** : Analysis of survival and incidence of nonneoplastic lesions was performed. Urine data was analyzed by nonparametric methods.

**Remark** : Control group treatment: 50 male and 50 female rats exposed to air only

**Result** : Survival of all exposed groups was similar to the chamber controls. There were no exposure-related changes in clinical observations or body weights. The incidences of splenic fibrosis in the 700 and 7,000 ppm males (24/50, 22/50) were significantly greater than that in the chamber control group (11/50).

**Test condition** : Groups of 50 rats/sex/group (approx. 6 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 104

weeks. Individual clinical observations were recorded initially, monthly through week 89, and then every 2 weeks until the end of the study. Individual body weights were recorded initially, monthly through week 91, and then every 2 weeks until the end of the study. Urine samples were collected 3, 6, 12, and 18 months from 10 rats/sex/group and analyzed for urine weight, creatinine, and vinyl lactic acid (a metabolite of isoprene). After 104 weeks of exposure, necropsies were performed on all rats and all major tissues preserved. Histopathologic examinations were performed on all tissues from all study animals. No blood analyses or organ weights were performed.

**Test substance** : Isoprene (CAS# 78-79-5) purity > 99.7%.
**Conclusion** : In this study survival of all exposed groups were similar to the chamber controls. There were no exposure related changes in clinical observations or body weights.
**Reliability** : (1) valid without restriction
11.01.2005 (54)

**Type** :
**Species** : Mouse
**Sex** : male/female
**Strain** : B6C3F1
**Route of admin.** : Inhalation
**Exposure period** : 4 or 8 hours/day
**Frequency of treatm.** : variable - 5 days/week for 20, 40 or 80 weeks
**Post exposure period** : Variable - animals held following exposures until week 96 or 105
**Doses** : 0, 10, 70, 140, 280, 700, 2200 ppm
**Control group** : Yes
**NOAEL** : = 10 ppm
**LOAEL** : = 70 ppm
**Method** : other
**Year** : 1996
**GLP** : Yes
**Test substance** : other TS: Isoprene

**Method** : Body weights, organ weights and hematology data were evaluated by analysis of variance (ANOVA) followed by Duncan's new multiple range test.
**Remark** : Control group and treatment: 50 male and 50 female mice exposed to air only
**Result** : Exposure of mice to the varied concentrations and schedules did not produce any significant signs of general toxicity. There was a concentration-related effect on survival due to increases in selected tumor development and associated mortality. Survival was near or below 50% after 95 weeks for mice exposed >280 ppm for 80 weeks - surviving mice in these groups were necropsied during week 96. In the micronucleus evaluation, the mean incidence of micronuclei in peripheral blood was significantly increased at 700 ppm and higher after 80 weeks, and at 2200 ppm after 40 weeks.
**Test condition** : Twelve groups of 50 male mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm for 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period until week 105. Three groups of 50 female mice were exposed to 0, 10, and 70 ppm for 8 hours/day for 80 weeks and also held for observation until week 105. Clinical observations and body weights were recorded weekly for 13 weeks and then monthly. Hematology and micronucleus evaluations were performed on 10 mice/group at 40 and 80 weeks. Complete histopathology evaluations were performed on organs and tissues from all mice.
**Test substance** : Isoprene (CAS# 78-79-5) purity > 99.0%.
**Conclusion** : In this study no significant signs of general toxicity were observed. However, a concentration related effect on survival was observed.

**Reliability** : (1) valid without restriction
29.07.2005 (61)

**Type** :
**Species** : Mouse
**Sex** :
**Strain** : Wistar
**Route of admin.** : Inhalation
**Exposure period** : 4 months
**Frequency of treatm.** : 5 days/week, 4 hours/day
**Post exposure period** : 1 month
**Doses** : 0.011 and 0.12 mg/l
**Control group** : no data specified

**Result** : No data on number of animals.

In the low dose group no changes during treatment observed. After the 1 month post observation period the mitotic index in the thymus was significantly raised.

In the high dose group during several phases of the treatment the cell counts of the thymus were significantly increased or decreased. Simultaneously, the thymus weight was significantly increased or decreased. The mitotic index was decreased, but came to a normalized level during the posttreatment observation of 1 month.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
29.07.2005 (44)

**Type** :
**Species** : Rat
**Sex** : no data
**Strain** : Fischer 344
**Route of admin.** : Inhalation
**Exposure period** : 6 months
**Frequency of treatm.** : 5 days/week, 4 hours/day
**Post exposure period** : yes, animals held for 6 months prior to sacrifice
**Doses** : 0, 70, 220, 700, 2200, 7000 ppm
**Control group** : Yes
**Method** :
**Year** :
**GLP** : Yes
**Test substance** :

**Result** : 12% mortality in 7000 ppm group

Rats showed no effects other than marginal increased incidence of interstitial cell benign adenoma, a common spontaneous lesion in this strain.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
11.01.2005 (55)

**Type** :
**Species** : Mouse
**Sex** : no data
**Strain** : B6C3F1
**Route of admin.** : Inhalation
**Exposure period** : 6 months

| | | |
|---|---|---|
| **Frequency of treatm.** | : | 5 days/week, 4 hours/day |
| **Post exposure period** | : | yes, animals held for 6 months prior to sacrifice |
| **Doses** | : | 0, 70, 220, 700, 2200, 7000 ppm |
| **Control group** | : | Yes |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | Yes |
| **Test substance** | : | |
| **Result** | : | 12% mortality in 7000 ppm group |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |

11.01.2005 (55)

| | | |
|---|---|---|
| **Type** | : | |
| **Species** | : | Rat |
| **Sex** | : | |
| **Strain** | : | |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 5 months |
| **Frequency of treatm.** | : | 4 hours/day |
| **Post exposure period** | : | No |
| **Doses** | : | 2.2 - 4.9 mg/l air in the inhalation chamber |
| **Control group** | : | no data specified |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | No |
| **Test substance** | : | |
| **Remark** | : | No data on control groups. |
| **Result** | : | The studies mainly showed a depression of the lymphatic system and after 3 months a reduced O2-consumption.<br><br>Histopathological findings: irritation of the bronchia, lung damages, irritation of the thyroid gland |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |

29.07.2005 (24)

| | | |
|---|---|---|
| **Type** | : | |
| **Species** | : | Mouse |
| **Sex** | : | |
| **Strain** | : | |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 4 months |
| **Frequency of treatm.** | : | 4 hours/day |
| **Post exposure period** | : | No |
| **Doses** | : | 2.2 - 4.9 mg/l air in the inhalation chamber |
| **Control group** | : | no data specified |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | No |
| **Test substance** | : | |
| **Remark** | : | No data on control groups. |
| **Result** | : | Histopathological findings: degenerative changes in the liver |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |

29.07.2005 (24)

| | | |
|---|---|---|
| **Type** | : | |
| **Species** | : | Rabbit |
| **Sex** | : | |
| **Strain** | : | |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 4 months |
| **Frequency of treatm.** | : | 4 hours/day |
| **Post exposure period** | : | No |
| **Doses** | : | 2.2 - 4.9 mg/l air in the inhalation chamber |
| **Control group** | : | no data specified |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | No |
| **Test substance** | : | |
| **Remark** | : | No data on control groups. |
| **Result** | : | At the end of the treatment the rabbits showed increased leucocyte counts, decreased erythrocyte counts and increased organ weights.<br><br>Histopathological findings: damage of the myocard |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |

29.07.2005 (24)

## 5.5 GENETIC TOXICITY 'IN VITRO'

| | | |
|---|---|---|
| **Type** | : | Ames test |
| **System of testing** | : | Bacterial |
| **Test concentration** | : | 0, 100, 333, 1000, 3333, 10000 µg/plate |
| **Cycotoxic concentr.** | : | |
| **Metabolic activation** | : | with and without |
| **Result** | : | Negative |
| **Method** | : | EPA OTS 798.5265 |
| **Year** | : | 1986 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | A positive response was defined as a reproducible, dose-related increase in revertant colonies in any one strain/activation combination. There was no minimum percentage or fold increase required for the chemical to be judged positive or weakly positive. |
| **Remark** | : | Species/Strain: Salmonella / TA98, TA100, TA1535, TA1537<br>Species and cell type: Rat and hamster liver S9 fraction<br>Quantity: 0.5 ml/plate<br>Induced or not induced: Arochlor 1254-induced (500 mg/kg for 5 days) |
| **Result** | : | Isoprene was not mutagenic in any of the five strains of Salmonella tested in the presence or absence of Aroclor-induced rat or hamster liver S9. There was no dose-related reproducible increase in the number of revertants in any of the 4 strains tested. |
| **Test condition** | : | The preincubation modification of the Salmonella/mammalian microsome assay was used to test isoprene in five different Salmonella strains in the presence and absence of rat and hamster liver S-9. Five dose levels were tested , with three plates per dose level. The high dose was limited by toxicity to 10,000 ug/plate. Concurrent positive controls were also tested with and without metabolic activation. The positive control substances used were: sodium azide for TA1535 and TA100; 4-nitro-o-phenylenediamine for |

TA98; and 9-aminoacridine for TA1537; 2-aminoanthracene was used with all strains with hamster and rat liver metabolic activation systems. The assay was repeated less than one week after completion of the initial test.

**Test substance** : Isoprene (CAS# 78-79-5) purity > 99%.
**Conclusion** : Isoprene was not mutagenic in the Ames Salmonella mutagenicity test.
**Reliability** : (1) valid without restriction
Evaluated as part of a NTP-sponsored interlaboratory study of 270 chemicals.

29.07.2005 (51)

**Type** : Ames test
**System of testing** : Salmonella typhimurium TA 98, 100, 1530, 1535, 1538
**Test concentration** : 25 %v/v in the atmosphere, for TA 1538 up to 75 %v/v
**Cycotoxic concentr.** :
**Metabolic activation** : with and without
**Result** : Negative
**Method** :
**Year** :
**GLP** : no data
**Test substance** :

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (13)

**Type** : Ames test
**System of testing** : Salmonella typhimurium TA 102, 104
**Test concentration** :
**Cycotoxic concentr.** :
**Metabolic activation** : Without
**Result** : Negative
**Method** :
**Year** :
**GLP** : no data
**Test substance** :

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (39) (52)

**Type** : other: Ames test with metabolites of isoprene
**System of testing** : Salmonella typhimurium TA 98, 100
**Test concentration** : 136 - 2043 µg/plate
**Cycotoxic concentr.** :
**Metabolic activation** : Without
**Result** : Ambiguous
**Method** :
**Year** :
**GLP** : no data
**Test substance** :

**Remark** : Tested metabolites:
(I) 3,4-Epoxy-3-methyl-1-butene
(II) 3,4-Epoxy-2-methyl-1-butene
(III) 2-Methyl-1,2,3,4-diepoxybutane

I and II: no mutagenic properties, but at 2043 µg/plate cytotoxic effects
III: dose-dependent increased number of revertants

III has strong alkylating properties

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (23)

**Type** : other: Ames bacterial reverse mutation test
**System of testing** : Enclosed static vapor phase exposure chambers
**Test concentration** : 0, 0.25 to 25% isoprene (2,500 to 250,000 ppm) in vapor phase
**Cycotoxic concentr.** :
**Metabolic activation** : with and without
**Result** : Negative
**Method** : other: Similar to methods described in OECD 471, and by Green (1984)
**Year** : 2003
**GLP** :
**Test substance** : other TS: Isoprene (CAS No. 78-79-5) Purity 99%

**Method** : A positive response is defined as reproducible, dose-related increase in revertant colonies in any one strain. An increase of 2x over background is required for a positive response in Salmonella strains TA98 & TA100 and for E. coli bacteria, and 3x background for Salmonella TA1535 & TA1537.
**Remark** : Species/Strain: Salmonella typhimurium TA98, TA100, TA1535, TA1537, Escherichia coli WP2 uvrA (pKM101)

Species and Cell Type: B6C3F1 mouse liver S9 and microsomal fractions. Source - Molecular Toxicology, Boone, North Carolina

Quantity: 0.24 & 0.47 mg protein (microsomes), and 1.67 mg protein (S-9) added per plate
**Result** : Negative. Based upon lack of dose-responsiveness and relative revertant scores <2.
The highest increases of isoprene-induced revertant rates relative to background were 1.3-1.7 in TA 1535, and 0.6-1.6 in E. coli, both in presence of S-9. The positive control, VC, induced relative rates of 22-34, and 4.4-6.3, respectively, in these assays. Due to lack of dose-responsiveness, and the fact all revertant rates for isoprene were < 1.7, the criteria for a positive response were not met.
**Test condition** : The Salmonella/mammalian liver enzyme assay was used to test isoprene in four Salmonella & one E. coli strain in the presence of mouse liver S-9 and microsomes. Isoprene vapor exposures above 25% posed significant cytotoxicity based upon a pilot study with exposures up to 100% isoprene. Exposure levels were tested in three plates per dose level. The concurrent positive control, vinyl chloride monomer (VC), was tested at 2% to confirm both bacterial mutagenic and metabolic activating capacities for liver enzymes.
**Test substance** : other TS: Isoprene (CAS No. 78-79-5) Purity 99%
**Conclusion** : Isoprene was not mutagenic in the Ames bacterial mutagenicity test.
**Reliability** : (1) valid without restriction
Evaluated at Huntingdon Life Sciences (UK) as part of IISRP-sponsored study of olefinic chemicals.

10.12.2003 (26) (32)

**Type** : Sister chromatid exchange assay
**System of testing** : Chinese hamster ovary (CHO) cells
**Test concentration** : 50, 160, 500, 1600 ug/ml (without S9), or 160, 500, 1600, 5000 ug/ml (with S9)
**Cycotoxic concentr.** :
**Metabolic activation** :
**Result** : Negative
**Method** : OECD Guide-line 479
**Year** : 1987

| | | |
|---|---|---|
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points. A frequency 20% above the solvent control group was considered positive. Positive trend tests ($p<0.05$) in the absence of a significant difference at any one dose were considered equivocal. |
| **Remark** | : | Induced or not induced: Aroclor 1254-induced Sprague-Dawley rat liver S9.<br>Control groups and treatment: Solvent controls -- dimethylsulfoxide; positive controls -- Mitomycin-C (without S9), cyclophosphamide (with S9). |
| **Result** | : | No increases in SCEs were noted in cultured CHO cells treated with isoprene, with or without S9. |
| **Test condition** | : | Isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCE) both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and four doses of isoprene. A single flask per dose was used. All slides were scored blind and those from a single test were read by the same person. Fifty 2nd-division metaphase cells were scored for frequency of SCEs/cell from each dose level. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 99%. |
| **Conclusion** | : | Isoprene did not induce sister chromatid exchanges in vitro in cultures of Chinese hamster ovary cells. |
| **Reliability** | : | (1) valid without restriction<br>Evaluated as part of a NTP-sponsored study of 108 chemicals. |

29.07.2005 (21)

| | | |
|---|---|---|
| **Type** | : | Chromosomal aberration test |
| **System of testing** | : | Chinese hamster ovary (CHO) cells |
| **Test concentration** | : | 1600, 3000, 5000 ug/ml |
| **Cycotoxic concentr.** | : | |
| **Metabolic activation** | : | |
| **Result** | : | Negative |
| **Method** | : | OECD Guide-line 473 |
| **Year** | : | 1987 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points. A statistically significant ($p<0.05$) difference for one point and a significant trend ($p<0.015$) was considered positive. Positive trend tests ($p<0.05$) in the absence of a significant difference at any one dose were considered equivocal. |
| **Remark** | : | Induced or not induced: Aroclor 1254-induced Sprague-Dawley rat liver S9.<br>Control groups and treatment: Solvent control -- dimethylsulfoxide; positive controls -- Mitomycin-C (without S9), cyclophosphamide (with S9). |
| **Result** | : | No increases in chromosomal aberrations were noted in cultured CHO cells treated with isoprene, with or without S9. |
| **Test condition** | : | Isoprene was tested in cultured Chinese hamster ovary (CHO) cells for induction of chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and three doses of isoprene. A single flask per dose was used. All slides were scored blind and those from a single test were read by the same person. Two hundred 1st-division metaphase cells were scored for chromosomal aberrations at each dose level. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 99%. |

**Conclusion** : Isoprene did not induce chromosomal aberrations in vitro in cultures of Chinese hamster ovary cells.
**Reliability** : (1) valid without restriction
Evaluated as part of a NTP-sponsored study of 108 chemicals.
29.07.2005 (21)

## 5.6 GENETIC TOXICITY 'IN VIVO'

**Type** : Sister chromatid exchange assay
**Species** : Mouse
**Sex** : Male
**Strain** : B6C3F1
**Route of admin.** : Inhalation
**Exposure period** : 6 hours/day for 12 days
**Doses** : 0, 438, 1750, 7000 ppm
**Result** : Positive
**Method** : other
**Year** : 1988
**GLP** : Yes
**Test substance** : other TS: Isoprene

**Method** : The frequencies of sister chromatid exchanges (SCEs) were analyzed for increasing trend by the one-tailed Cochran-Armitrage trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using either the one-tailed or two-tailed t-test.
**Result** : NOAEL (NOEL): < 438 ppm
LOAEL (LOEL): 438 ppm

Exposure to isoprene for 6 h/day at 0, 438, 1750, or 7000 ppm for 12 days induced a significant increase in the frequency of SCEs in bone marrow cells at all three dose levels (4.40 at 0 ppm, 14.84 at 438 ppm, 11.61 at 1750 ppm, and 13.98 at 7000 ppm). The increased SCE responses in the exposed groups were not statistically different from each other.. There were no significant clinical signs or mortality throughout the study.
**Test condition** : Fifteen male B6C3F1 mice (approximately 6-7 weeks old) per group were exposed for 12 days, 6 h/day to 0, 438, 1750, or 7000 ppm of isoprene by inhalation. The exposure regimen was 3 exposure days, 2 days off, 5 exposure days, 2 days off, then 4 exposure days. Exposure concentrations were monitored by gas chromatography. The animals were implanted with a BrdU tablet 1 hour before the 12th exposure. Two hours before sacrifice on the following day, the animals received an intraperitoneal injection of colchicine. For analysis of SCE, 5 mice per exposure group were killed 24 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Twenty-five second-division metaphase cells were scored for SCEs from 4 mice/group.
**Test substance** : Isoprene (CAS# 78-79-5) purity > 98%.
**Conclusion** : Isoprene was found to be genotoxic and cytotoxic to mouse bone marrow in vivo - inducing SCE, inhibiting cellular proliferation, and suppressing the rate of erythropoiesis. The lack of significant difference in SCEs among the three exposed groups suggests a saturation of the metabolic capacity of male mice to form reactive species.
**Reliability** : (1) valid without restriction
NTP-sponsored study.
10.12.2003 (73)

**Type** : other: Mammalian Bone Marrow Chromosomal Aberration Test
**Species** : Mouse

| | | |
|---|---|---|
| **Sex** | : | Male |
| **Strain** | : | B6C3F1 |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 6 hours/day for 12 days |
| **Doses** | : | 0, 438, 1750, 7000 ppm |
| **Result** | : | Negative |
| **Method** | : | OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis" |
| **Year** | : | 1988 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | The frequencies of chromosomal aberrations (Abs) were analyzed for increasing trend by the one-tailed Cochran-Armitrage trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using either the one-tailed or two-tailed t-test. |
| **Result** | : | NOAEL (NOEL): 7000 ppm<br>LOAEL (LOEL): > 7000 ppm<br><br>Exposure to isoprene for 6 h/day at 0, 438, 1750, or 7000 ppm for 12 days did not induce a statistically significant increase in the frequency of chromosomal aberrations (Abs) in bone marrow cells. The incidence of bone marrow cells with chromosomal aberrations (Abs) was slightly elevated in the exposed groups compared to the control (0.02 at 0 ppm vs. 0.04, 0.05, and 0.04 at 438, 1750, and 7000 ppm), but these increases were not statistically significant. Mitotic index data indicated no significant change in the percentage of bone marrow cells engaged in division, although the 7000 ppm group was slightly increased compared to the contols (1.15% vs 1.30%). Analysis of average generation time showed a statistically significant lengthening of the cell cycle duration of proliferating cells in the 7000 ppm group (13.72 hours at 7000 ppm vs.11.68 hours at 0 ppm). |
| **Test condition** | : | Fifteen male B6C3F1 mice (approximately 6-7 weeks old) per group were exposed for 12 days, 6 h/day to 0, 438, 1750, or 7000 ppm of isoprene by inhalation. The exposure regimen was 3 exposure days, 2 days off, 5 exposure days, 2 days off, then 4 exposure days. Exposure concentrations were monitored by gas chromatography. The animals were implanted with a BrdU tablet 1 hour before the 12th exposure. Two hours before sacrifice on the following day, the animals received an intraperitoneal injection of colchicine. For analysis of Abs, 10 mice per exposure group were killed 17-20 hours after BrdU implantation. Bone marrow was removed, fixed onto slides, and stained using differential chromatid staining. Fifty first-division metaphase cells were scored for Abs from 8 mice/group. Additionally, 100 randomly selected metaphase cells per slide were scored for replication history to provide data on cell generation time, a measure of cell proliferation kinetics. The percentage of cells in metaphase among 1000 cells/sample was used to calculate the mitotic index. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 98%. |
| **Conclusion** | : | The incidence of bone marrow cells with chromosomal aberrations in male mice treated with isoprene for 12 days were slightly elevated at all dose groups compared to the controls, but were not statistically increased. |
| **Reliability** | : | (1) valid without restriction<br>NTP-sponsored study. |

04.04.2003 (73)

| | | |
|---|---|---|
| **Type** | : | other: Mammalian Erythrocyte Micronucleus Test |
| **Species** | : | Mouse |
| **Sex** | : | Male |
| **Strain** | : | B6C3F1 |

| | | |
|---|---|---|
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 6 hours/day for 12 days |
| **Doses** | : | 0, 438, 1750, 7000 ppm |
| **Result** | : | Positive |
| **Method** | : | OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test" |
| **Year** | : | 1988 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | The number of micronucleated erythrocytes (MN) were summed across animals within each group and analyzed for increasing trend by a one-tailed trend test ($p<0.05$). For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent control were performed using a one-tailed Pearson Chi square test to determine the minimal effective dose. |
| **Result** | : | NOAEL (NOEL): < 438 ppm<br>LOAEL (LOEL): 438 ppm<br><br>Exposure to isoprene for 6 h/day at 0, 438, 1750, or 7000 ppm for 12 days induced a statistically significant increase in the frequency of MN-PCEs and NCEs in male mice at all exposure levels tested. The frequencies of MN-PCEs were 2.00, 12.00, 15.60, and 16.93 at 0, 438, 1750, and 7000 ppm. The responses at the 1750 and 7000 ppm levels both were greater than the 438 ppm level, but not statistically different from each other. There also was a dose-related decrease in the percentage of PCEs, a measure of the rate erythropoiesis (3.91, 3.00, 2.87, and 1.64 at 0, 438, 1750, and 7000 ppm). There were no significant clinical signs or mortality throughout the study. |
| **Test condition** | : | Approximately 24 hours following the last exposure peripheral blood samples were obtained from each animal by tail snip, immediately air-dried and fixed with methanol. One thousand polychromatic erythrocytes (PCEs) and 1000 normochromatic erythrocytes (NCEs) were scored per animal for frequency of micronucleated erythrocytes (MN). The percentage of PCEs in 1000 erythrocytes was also determined as a measure of isoprene-induced toxicity. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 98%. |
| **Conclusion** | : | Isoprene was found to be genotoxic to mouse bone marrow in vivo by inducing increased MN in the peripheral blood of male mice. Suppression of erythropoiesis was suggested by decreased percentage of PCEs. |
| **Reliability** | : | (1) valid without restriction<br>NTP-sponsored study. |

07.01.2005 (73)

| | | |
|---|---|---|
| **Type** | : | other: Rat Lung Fibroblast Micronucleus Test |
| **Species** | : | Rat |
| **Sex** | : | male/female |
| **Strain** | : | Fischer 344 |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 6 hours/day, 5 days/week for 4 weeks |
| **Doses** | : | 0, 220, 700, or 7000 ppm |
| **Result** | : | Negative |
| **Method** | : | other |
| **Year** | : | 1997 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Means, standard deviations, and standard error of the mean for the number of mononucleated cells/1000 binucleated cells and micronuclei/1000 binucleated cells were calculated. A two-way analysis of variance was used to analyze the measurements. Intergroup differences were delineated by |

Tukey's studentized range test.

**Result** : There were no statistically significant differences between the male or female exposed and control groups for micronucleated rat lung fibroblasts. There were no significant clinical signs or mortality during the exposure period.

**Test condition** : This study was performed in conjunction with a two-year carcinogenicity study. Groups of 10 male and 10 female rats (approximately 6-7 weeks old) per group were exposed for 4 weeks (17-19 total exposures) to 0, 220, 700, or 7000 ppm of isoprene by inhalation. The rats received at least two consecutive days of exposure prior to sacrifice and lung cell isolation. Lung fibroblasts were isolated and cultured in single-chamber slides for 72 hours. The slides were fixed and stained (acridine orange), and 1000 binucleated cells on each of two slides per animal were scored. The number of mononucleated cells and micronuclei were recorded following a standard scoring criteria.

**Test substance** : Isoprene (CAS# 78-79-5) purity > 99.7%.

**Conclusion** : No significant increase in the frequency of micronucleated lung fibroblasts was observed in male and female rats exposed to isoprene for 4 weeks.

**Reliability** : (1) valid without restriction
Non-standard method, but comparable to guideline study. Conducted as part of NTP two-year carcinogenicity study.

11.01.2005 (54)

**Type** : Micronucleus assay
**Species** : Mouse
**Sex** : male/female
**Strain** : B6C3F1
**Route of admin.** : Inhalation
**Exposure period** : 20, 40 or 80 weeks
**Doses** : 0, 10, 70, 140, 280, 700, 2200 ppm/ 4 or 8 hours/day, 5
**Result** :
**Method** :
**Year** :
**GLP** : Yes
**Test substance** : as prescribed by 1.1 - 1.4

**Remark** : Induction of micronuclei after chronic inhalation exposure.
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

08.04.2003 (62)

**Remark** : Refers to the study that was described above.
**Result** : When the results of this study were compared to results for butadiene using the same end points, route of exposure and strain, species and sex of animals, isoprene was considered to be of lower genetic toxicity despite being tested at substantially higher concentrations.
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (65)

## 5.7 CARCINOGENICITY

**Species** : Rat
**Sex** : male/female
**Strain** : Fischer 344
**Route of admin.** : Inhalation

| | | |
|---|---|---|
| **Exposure period** | : | 6 hours/day |
| **Frequency of treatm.** | : | 5 days/week for 104 weeks |
| **Post exposure period** | : | None |
| **Doses** | : | 0, 220, 700, or 7000 ppm |
| **Result** | : | Positive |
| **Control group** | : | Yes |
| **Method** | : | other |
| **Year** | : | 1997 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Analysis of survival and incidence of neoplastic and nonneoplastic lesions was performed. |
| **Remark** | : | Control group treatment: 50 male and 50 female rats exposed to air only. |
| **Result** | : | The incidences of mammary gland fibroadenoma in 7,000 ppm males (7/50) and in all groups of exposed females (12/50, 19/50, 17/50) were significantly greater than those in the chamber control groups (1/50 males, 7/50 females). The incidences of renal tubule adenoma in 7,000 ppm males (6/50) and of renal tubule hyperplasia in 700 ppm and 7,000 ppm males (6/50, 8/50) were significantly greater than those in the chamber controls (0/50). The severity of kidney nephropathy was slightly increased in 7,000 ppm males when compared to chamber controls. An exposure-related increase in the incidences of interstitial cell adenoma of the testis was observed in male rats (33/50, 37/50, 44/50, 48/50). The incidences of bilateral interstitial cell adenoma and of unilateral and bilateral interstitial cell adenoma (combined) of the testis in the 700 ppm and 7,000 ppm (37/50, 48/50) males were significantly greater than in the chamber controls (20/50). Single incidences of several rare neoplasms including benign astrocytoma, malignant glioma, malignant medulloblastoma, benign meningeal granular cell tumor, and meningeal sarcoma were observed in the brains of female rats in all three exposure groups. The incidences of splenic fibrosis in the 700 and 7,000 ppm males (24/50, 22/50) were significantly greater than that in the chamber control group (11/50). |
| **Test condition** | : | Groups of 50 rats/sex /group (approx. 6 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for 104 weeks. Individual clinical observations were recorded initially, monthly through week 89, and then every 2 weeks until the end of the study. Individual body weights were recorded initially, monthly through week 91, and then every 2 weeks until the end of the study. Urine samples were collected 3, 6, 12, and 18 months from 10 rats/sex/group and analyzed for urine weight, creatinine, and vinyl lactic acid (a metabolite of isoprene). After 104 weeks of exposure, necropsies were performed on all rats and all major tissues preserved. Histopathologic examinations were performed on all tissues from all study animals. No blood analyses or organ weights were performed. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 99.7%. |
| **Conclusion** | : | Isoprene exposures were associated with increases in rates of benign tumors in the testes and kidney (male), and mammary gland (male and female). No significant increases were seen for malignant tumors in this study. For this reason, and the fact that brain tumors in females were of several distinct cell types, the overall level of evidence presented for the carcinogenicity of isoprene in rats is, at most, limited. However, the NTP concluded that: there was clear evidence of carcinogenic activity in male rats based on increased incidences of mammary gland fibroadenoma and carcinoma, renal tubule adenoma, and testicular interstitial cell adenoma; some evidence of carcinogenic activity in female rats based on increased incidences and multiplicity of mammary galnd fibroadenoma. A low incidence of rare brain neoplasms in exposed female rats may have been due to exposure to isoprene. |
| **Reliability** | : | (1) valid without restriction |

11.01.2005 (54)

| | | |
|---|---|---|
| **Species** | : | Mouse |
| **Sex** | : | male/female |
| **Strain** | : | B6C3F1 |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 4 or 8 hours/day |
| **Frequency of treatm.** | : | Variable - 5 days/week for 20, 40 or 80 weeks |
| **Post exposure period** | : | Variable - animals held following exposures until week 96 or 105 |
| **Doses** | : | 0, 10, 70, 140, 280, 700, or 2200 ppm |
| **Result** | : | Positive |
| **Control group** | : | Yes |
| **Method** | : | other |
| **Year** | : | 1996 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: isoprene |
| **Method** | : | Body weights, organ weights and hematology data were evaluated by analysis of variance (ANOVA) followed by Duncan's new multiple range test. Incidences of tumor types were analyzed using Fischer's exact test applied to each combination of exposure group and tumor type. |
| **Remark** | : | Control group and treatment: 50 male and 50 female mice exposed to air only. |
| **Result** | : | The carcinogenic potential of isoprene was evaluated as a function of concentration, length of daily exposure, and weeks of exposure as independent variables. Exposure of mice to the varied concentrations and schedules did not produce any significant signs of general toxicity. There was a concentration-related effect on survival due to increases in selected tumor development and associated mortality. Survival was near or below 50% after 95 weeks for mice exposed >280 ppm for 80 weeks - surviving mice in these groups were necropsied during week 96. Isoprene exposure caused an increase in neoplasms of the lung, liver, Harderian gland, forestomach, lymphoreticular system of male mice and in the Harderian gland and pituitary gland of female mice at concentrations of 70 ppm and higher. The product of concentration and length/duration of exposure was not a sufficient basis for prediction of tumor risk. In the micronucleus evaluation, the mean incidence of micronuclei in peripheral blood was significantly increased at 700 ppm and higher after 80 weeks, and at 2200 ppm after 40 weeks (the 280 and 700 ppm groups were not sampled by protocol design). |
| **Test condition** | : | Twelve groups of 50 male mice were exposed to 0, 10, 70, 140, 280, 700, or 2200 ppm for 4 or 8 hours/day, 5 days/week for 20, 40, or 80 weeks followed by a holding period until week 105. Three groups of 50 female mice were exposed to 0, 10, and 70 ppm for 8 hours/day for 80 weeks and also held for observation until week 105. Clinical observations and body weights were recorded weekly for 13 weeks and then monthly. Hematology and micronucleus evaluations were performed on 10 mice/group at 40 and 80 weeks. Complete histopathology evaluations were performed on organs and tissues from all mice. |
| **Conclusion** | : | The results of this study indicated that concentration, , length of daily exposure, and weeks of exposure did not affect tumor incidence equivalently and total cumulative exposure was not sufficient for predicting oncogenic risk from isoprene exposure in mice. There appeared to be threshold for oncogenic effects in mice, which varied by organ and tumor type. For male mice, the LOEL was 700 ppm for lung tumor and hemangiosarcoma, 280 ppm for malignant forestomach tumors and histiocytic sarcomas, 140 ppm for liver tumors, and 70 pmm for Harderian gland tumors. For female mice, the LOEL was 70 ppm for total non-liver, non-lung adenomas and possibly for hemagiosarcomas. |
| **Reliability** | : | (1) valid without restriction |

29.07.2005 (61)

| | | |
|---|---|---|
| **Species** | : | Mouse |
| **Sex** | : | Female |
| **Strain** | : | ICR |
| **Route of admin.** | : | Dermal |
| **Exposure period** | : | 18 weeks after a 3 week initiation with DMBA |
| **Frequency of treatm.** | : | 5 times/week |
| **Post exposure period** | : | no information |
| **Doses** | : | 1.5 % isoprene and 0.04 % crotone oil (mixture) |
| **Result** | : | |
| **Control group** | : | yes, concurrent vehicle |
| **Method** | : | other: initiation/promotion study |
| **Year** | : | 1971 |
| **GLP** | : | No |
| **Test substance** | : | |
| **Remark** | : | Study inadequate to assess the carcinogenic effects of isoprene. |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |

29.07.2005 (64)

| | | |
|---|---|---|
| **Species** | : | Mouse |
| **Sex** | : | male/female |
| **Strain** | : | B6C3F1 |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 40 or 80 weeks |
| **Frequency of treatm.** | : | 4 or 8 hours/day, 5 days/week |
| **Post exposure period** | : | 24 weeks after 80 weeks treatment->necropsy |
| **Doses** | : | 0, 10, 70, 140, 280, 700, 2200 ppm |
| **Result** | : | |
| **Control group** | : | Yes |
| **Method** | : | other |
| **Year** | : | |
| **GLP** | : | Yes |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | Study designed to determine the oncogenic potential as a function of exposure level, length of daily exposure and exposure duration. To achieve this 15 groups of 50 animals each were exposed to various concentrations of isoprene (0-2200 ppm), 4 to 8 hours daily for 40 or 80 weeks. There was an exposure related increase in tumours at multiple sites. The tumour pattern was similar to the profile observed in butadiene, 1,3- with the exception of the early onset of T-cell lymphoma in butadiene treated mice. Biostatistical analyses indicated, that the product of concentration and length/duration of exposure was not a sufficient basis for predicting tumour risk at any site. Extrapolation of tumour probability between the high and low concentrations based on cumulative exposure was not appropriate and could not be justified by statistical models. |
| **Source** | : | Deutsche Shell Chemie GmbH Eschborn<br>Exxon Chemical Europe Inc. Bruxelles |

11.01.2005 (62)

### 5.8.1 TOXICITY TO FERTILITY

| | | |
|---|---|---|
| **Type** | : | other: 13-week inhalation study |
| **Species** | : | Rat |
| **Sex** | : | male/female |
| **Strain** | : | Fischer 344 |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 6 hours/day |
| **Frequency of treatm.** | : | 5 days/week |
| **Premating exposure period** | | |
| **Male** | : | |
| **Female** | : | |
| **Duration of test** | : | 13 weeks |
| **No. of generation studies** | : | |
| **Doses** | : | 0, 70, 220, 700, 2200 or 7000 ppm |
| **Control group** | : | Yes |
| **NOAEL parental** | : | = 2200 ppm |
| **Method** | : | other |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Analysis of incidence of neoplastic and nonneoplastic lesions was performed. |
| **Remark** | : | Control group and treatment: 10 male and 10 female rats exposed to air only. |
| **Result** | : | There were no exposure-related effects in rats except a slight increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7000 ppm group. |
| **Test condition** | : | Groups of 10 animals/sex/group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Sperm motility and vaginal cytology were performed on all rats exposed to 0, 70, 700 or 7000 ppm isoprene. Histopathologic evaluations of the reproductive organs were performed on all rats as part of the terminal sacrifice for the core 13-week subchronic inhalation study. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 99%. |
| **Conclusion** | : | No significant effects on reproductive endpoints were observed in rats except slight changes in the testis at the highest exposure level (7000 ppm). |
| **Reliability** | : | (2) valid with restrictions<br>Limited reproductive toxicity data obtained as part of a NTP-sponsored subchronic inhalation toxicity study. |

29.07.2005 (49)

| | | |
|---|---|---|
| **Type** | : | other: 13-week inhalation study |
| **Species** | : | Mouse |
| **Sex** | : | male/female |
| **Strain** | : | B6C3F1 |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | 6 hours/day |
| **Frequency of treatm.** | : | 5 days/week |
| **Premating exposure period** | | |
| **Male** | : | |
| **Female** | : | |
| **Duration of test** | : | 13 weeks |
| **No. of generation studies** | : | |
| **Doses** | : | 0, 70, 220, 700, 2200 or 7000 ppm |

| | | |
|---|---|---|
| **Control group** | : | Yes |
| **NOAEL parental** | : | = 220 ppm |
| **Method** | : | other |
| **Year** | : | 1994 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Method** | : | Analysis of incidence of neoplastic and nonneoplastic lesions was performed. |
| **Remark** | : | Control group and treatment: 10 male and 10 female mice exposed to air only. |
| **Result** | : | In this study the testicular weight of male mice was reduced 35% in the 7000 ppm group, and morphological changes (seminiferous tubular atrophy) were detected in 2/10 mice. Males in the 700 and 7000 ppm groups had 12% and 30% lower epididymal weights, 12% and 46% lower spermatid head counts, 12% and 46% lower sperm concentrations, and 6% and 23% reductions in sperm motility, respectively. The female mice exposed to 7000 ppm exhibited estrous cycle lengths significantly longer than the control group (4.8 vs. 4.2 days). |
| **Test condition** | : | Groups of 10 animals /sex /group/species (6-8 weeks age at study initiation) were exposed to various levels of isoprene for 6 hrs/day, 5 days/week for thirteen weeks. Sperm motility and vaginal cytology were performed on all mice exposed to 0, 70, 700 or 7000 ppm isoprene. Histopathologic evaluations of the reproductive organs were performed on all and mice as part of the terminal sacrifice for the core 13-week subchronic inhalation study. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 99%. |
| **Conclusion** | : | Mice exhibited significant effects at 700 ppm or higher, including increased estrous cycle length and testicular atrophy, and decreased epididymal weight, sperm head count, sperm concentration, and sperm motility. |
| **Reliability** | : | (2) valid with restrictions<br>Limited reproductive toxicity data obtained as part of a NTP-sponsored subchronic inhalation toxicity study. |

29.07.2005 (49)

| | | |
|---|---|---|
| **Type** | : | One generation study |
| **Species** | : | Rat |
| **Sex** | : | Female |
| **Strain** | : | Wistar |
| **Route of admin.** | : | oral unspecified |
| **Exposure period** | : | 4 days |
| **Frequency of treatm.** | : | not specified |
| **Premating exposure period** | | |
| **Male** | : | |
| **Female** | : | |
| **Duration of test** | : | |
| **No. of generation studies** | : | |
| **Doses** | : | 22, 380, 1900 mg/kg b. w. |
| **Control group** | : | |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no data |
| **Test substance** | : | |
| **Remark** | : | Female rats received the oral between day 9 and 12 of gestation. |
| **Result** | : | No indication of embryotoxic or teratogenic effects.<br><br>The fetusses showed slightly retarded ossification of the |

sternum and the occiput.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

04.04.2003 (74)

### 5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

| | | |
|---|---|---|
| **Species** | : | Rat |
| **Sex** | : | Female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | gestation days 6-19 |
| **Frequency of treatm.** | : | 6 hours/day |
| **Duration of test** | : | Females sacrificed on gestation day 20 |
| **Doses** | : | 0, 280, 1400, or 7000 ppm |
| **Control group** | : | other: yes (10 virgin females) |
| **NOAEL maternal tox.** | : | = 7000 ppm |
| **NOAEL teratogen.** | : | = 7000 ppm |
| **Method** | : | EPA OTS 798.4350 |
| **Year** | : | 1989 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Remark** | : | Control group and treatment: air-exposed only |
| **Result** | : | Maternal effects: Exposure of pregnant rats to these concentrations of isoprene did not result in apparent maternal toxicity. The only effect observed in the rat dams was an increased kidney to body weight ratio at the highest level (7000 ppm).<br><br>Embryo/fetal effects: In rats, there was no adverse effect on any reproductive index at any level and there was no increase in fetal malformations or variations. A slight, but not statistically significant, increase in the incidence of reduced vertebral ossifications (centra) was noted at 7000 ppm. |
| **Test condition** | : | Approximately 30 positively mated rats were exposed on days 6-19 of gestation. The day of plug or sperm detection was designated as day 0. Body weights were recorded throughout the study period, and uterine and fetal body weights were obtained at sacrifice. Implants were enumerated and their status recorded. Live fetuses were sexed and examined for gross, visceral, skeletal, and soft-tissue craniofacial defects. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 99.7%. |
| **Conclusion** | : | Pregnant Sprague-Dawley rats and their offspring exhibited no significant toxic effects of isoprene at any exposure level in this study. |
| **Reliability** | : | (1) valid without restriction<br>NTP-sponsored study. |

10.01.2005 (53)

| | | |
|---|---|---|
| **Species** | : | Mouse |
| **Sex** | : | Female |
| **Strain** | : | CD-1 |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | Gestation days 6-17 |
| **Frequency of treatm.** | : | 6 hours/day |
| **Duration of test** | : | Females sacrificed on gestation day 18 |
| **Doses** | : | 0, 280, 1400, or 7000 ppm |
| **Control group** | : | Yes |
| **NOAEL maternal tox.** | : | = 1400 ppm |
| **NOAEL teratogen.** | : | < 280 - ppm |

| | | |
|---|---|---|
| **Method** | : | EPA OTS 798.4350 |
| **Year** | : | 1989 |
| **GLP** | : | Yes |
| **Test substance** | : | other TS: Isoprene |
| **Remark** | : | Control group and treatment: air-exposed only |
| **Result** | : | Maternal effects: Exposure of Swiss (CD-1) mice to isoprene resulted in (from day 12 onward) significant reductions in maternal body weight, body weight gain during treatment, and in uterine weight for the 7000 ppm group. Liver to body weight ratios for pregnant mouse dams were significantly increased in the 1400 and 7000 ppm groups compared to the control group, and kidney to body weight ratios were significantly increased the 7000 ppm group.<br><br>Embryo/fetal effects: In mice, there was an exposure-related and statistically significant reduction in fetal body weights at the 280 ppm level for female fetuses and at the 1400 ppm level for male fetuses. No embryotoxicity in the form of increased intrauterine death was present at any exposure level. There was no significant increase in the incidence of fetal malformations or ossifications, although two fetuses with cleft palate were found, one in each of the two highest exposure groups (1400 and 7000 ppm). Cleft palates were not detected in the control group. Increased incidences of variations (supernumerary ribs) were observed in the exposed groups, although this skeletal variation is generally considered a secondary effect of maternal toxicity or stress and it's significance is unclear. The incidence of supernumerary ribs (percent of fetuses examined) was 20.1, 23.8, 33.6, and 40.3% at 0, 280, 1400, and 7000 ppm. |
| **Test condition** | : | Approximately 30 positively mated mice were exposed on days 6-17 of gestation. The day of plug or sperm detection was designated as day 0. Body weights were recorded throughout the study period, and uterine and fetal body weights were obtained at sacrifice. Implants were enumerated and their status recorded. Live fetuses were sexed and examined for gross, visceral, skeletal, and soft-tissue craniofacial defects. |
| **Test substance** | : | Isoprene (CAS# 78-79-5) purity > 99.7%. |
| **Conclusion** | : | Swiss (CD-1) mouse dams exhibited significant toxic effects only at the 7000 ppm level; however the offspring exhibited significant signs of toxicity, including reductions in fetal body weight which were statistically significant at the 280 ppm level (lowest isoprene level) for female fetuses and at the 1400 ppm level for male fetuses. |
| **Reliability** | : | (1) valid without restriction<br>NTP-sponsored study. |

10.01.2005 (53)

| | | |
|---|---|---|
| **Species** | : | Rat |
| **Sex** | : | male/female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | Inhalation |
| **Exposure period** | : | day 6-19 of gestation |
| **Frequency of treatm.** | : | 6 hours/day, 7 days/week |
| **Duration of test** | : | 12/14 days |
| **Doses** | : | 0, 280, 1400, 7000 ppm |
| **Control group** | : | Yes |
| **Method** | : | |
| **Year** | : | 1989 |
| **GLP** | : | no data |
| **Test substance** | : | |
| **Remark** | : | No adverse effect on the dams or on any reproductive index at any dose level. |

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
29.07.2005 (45) (46)

**Species** : Mouse
**Sex** : male/female
**Strain** : CD-1
**Route of admin.** : Inhalation
**Exposure period** : day 6-17 of gestation
**Frequency of treatm.** : 6 hours/day, 7 days/week
**Duration of test** : 12/14 days
**Doses** : 0, 280, 1400, 7000 ppm
**Control group** : Yes
**Method** :
**Year** : 1989
**GLP** : no data
**Test substance** :

**Remark** : 7000 ppm induced reduction in maternal weight gain; reduced fetal body weight at all dose levels. No fetal malformations except extra ribs at 7000 ppm.
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
29.07.2005 (45) (46)

### 5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

### 5.9 SPECIFIC INVESTIGATIONS

### 5.10 EXPOSURE EXPERIENCE

**Remark** : Toxic effects observed in humans:

catarrhal inflammation, subtrophic and atrophic processes in thr upper respiratory tract, deterioration of olfaction were noted in isoprene rubber production workers.. Incidence and degree was correlated with duration of occupation.
**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles
29.07.2005 (50) (63)

### 5.11 ADDITIONAL REMARKS

**Type** : Metabolism

**Remark** : Isoprene is metabolized via a similar mechanism as that for butadiene. However, unlike butadiene, the monoepoxide intermediates of isoprene are not mutagenic. Only the minor epoxide (20 % of the total) is further oxidized to the mutagenic diepoxide. Furthermore, liver microsomes from mice and hamsters showed a six-fold higher maximum metabolic

velocity (Vmax) than those from rats and rabbits (Longo et al., 1985). This species-difference was further demonstrated using a two-compartment model of isoprene pharmacokinetics. Both rats and mice exhibited saturation kinetics when exposed to isoprene at concentrations above 300 ppm. However, the Vmax in mice was determined to be 400 µmol/h/kg or more than three times that in rats (130 µmol/h/kg) implying a species-sensitivity to diepoxide formation in the mouse.Endogenous isoprene production rate was determined to be 1.9 and 0.4 µmol/h/kg in rats and mice, respectively (Peter et al., 1987).

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (41) (58)

**Type** : Metabolism

**Remark** : This paper describes comparative studies on the stereochemistry of the metabolism of isoprene that were carried out in vitro using liver microsomes from rats, mice, monkeys, dogs, rabbits and humans. Differences between strains and gender were also investigated. In the production f the isoprene monoepoxides, microsomes from the livers of the male Sprague-Dawley or Wistar rat showed an approximately 2:1 preference for the formation of (S)-2-(1-methylethenyl)oxirane compared with the (R)-enantiomer. No enantioselectivity was observed for mouse or rabbit. In contrast, liver microsomes from dog, monkey or male human preferentially formed (R)-2(1-methylethenyl)oxirane. There was no enantioselectivity observed with microsomes from female human liver. In conclusion, the significant differences between species in the in vitro metabolism of isoprene indicate that stereochemical and mechanistic data should be taken into account when evaluating the results of animal studies designed to assess the carcinogenic risks to humans that may be associated with exposure to isoprene.

29.07.2005 (70)

**Type** : Metabolism

**Remark** : This study evaluated the stereoselectivity of the in vitro conversion of isoprene by liver enzymes of rats and mice. Reaction mixtures (0.5 mL) containing rat or mouse liver microsomes ( 1nmol of cytochrome P450) along with buffer and other co-factors were incubated with isoprene (10 umol) for 130 minutes at 37 degrees C. The percentage of the monooxirane enantiomers formed by the enzymatic isoprene epoxidation were determined by complexation gas chromatography by the head-space technique.

Isoprene was epoxidized by cytochrome P450 of rats and mice to 2-isopropenyloxirane and 2-methyl-2-vinyloxirane with slight but different product enantioselectivity. Only with mouse liver microsomes was a distinct regioselectivity observed. Both monooxiranes were further epoxidized to 2-methyl-2,2'-bioxirane with substrate enantioselectivity, product diastereoselectivity, and with product enantioselectivity. The epoxide hydrolase-catalyzed hydrolysis with rat and mouse liver microsomes occurs with substrate enantioselectivity. The epoxide hydrolase-catalyzed hydrolysis with rat and mouse liver microsomes occurs with substrate enantioselectivity. A better kinetic resolution was found for 2-isopropenyloxirane than for 2-methyl-2-vinyloxirane. While 2(R)-isopropenyloxirane was conjugated preferentially with glutathione, catalyzed by glutathione S-transferase, no enantiomer differentiation takes place in the case of 2-methyl-2-vinyloxirane.

29.07.2005 (77)

**Type** : Metabolism

**Remark** : Isoprene is one of the main constituents of endogenous origin in exhaled human breath. In this study, breath samples were collected from fifty volunteers, 30 women and 20 men, aged 15 to 60 years, at various times of the day. Twenty-five of them also collected samples at different moments of the night, either while entirely awake or just after being awakened and allowed to fall asleep again. For practical purposes, samples obtained immediately after spontaneous or induced awakening were considered to be identical to those that might have been collected during sleep.
The concentration of isoprene in the breath taken at different moments of the daytime period , between 8 and 23 hours, from 50 healthy volunteers while fully awake was 14.6 + 6.4 nmol/L. This result is in agreement with other published values and demonstrates that the elimination of isoprene does not appreciably change during the diurnal period in individuals who stay awake. The isoprene concentration in the individuals awake during the night (i.e., 17.7 + 7.0 nmoles/L ) was similar to that in individuals awake during the day (14.6 + 6.4 nmoles/L). The isoprene concentration in the 13 subjects who were allowed to sleep was significantly higher than in the 9 subjects who stayed awake. Thus, in the absence of sleep during the night, the concentration of isoprene in the breath did not increase. On awakening in the morning or in the middle of the night, isoprene concentration was observed to fall sharply in less than 20 minutes. The isoprene concentration decreased from 42.4 + 13.5 to 18.2 + 4.7 nmoles/L (n=6) at 0200 hours and from 45.3 + 16.5 to 23.3 + 7.7 nmoles/L (n=14) at 0600 hours.
In conclusion, this study demonstrates that the concentration of isoprene varies with states of sleep and wakefulness, increasing during sleep and decreasing sharply just after awakening.

29.07.2005 (6)

**Type** : Metabolism

**Remark** : The present study investigated the metabolism of isoprene by the mouse liver cytochrome P-450 system to quantitatively determine the formation of possibly genotoxic epoxide intermediates. This study demonstrated that mouse liver microsomal mono-oxygenases metabolize isoprene to the corresponding mono-epoxides. The reaction was shown to be dependent on NADPH and oxygen and was inhibited by carbon monoxide, metyrapone and SKF52S-A. Of the two epoxides formed, 3,4-epoxy-3-methyl-1-butene was the major metabolite (approximately 80% formed) whereas 3,4-epoxy-2-methyl-1-butene was the minor metabolite (approximately 20% formed). The minor metabolite, 3,4-epoxy-2-methyl-1-butene was further epoxidated to the mutagenic and presumably carcinogenic isoprene diepoxide.

29.07.2005 (14)

**Type** : Toxicokinetics

**Remark** : The purpose of this study was to determine the toxicokinetics of inhaled isoprene in B6C3F1 mice and to compare the data to previously published toxicokinetic data inF344 rats. Male B6C3F1 mice were exposed to nominal concentrations of 20, 200, and 2000 ppm isoprene or [14C] isoprene for up to 6 hours. For all exposures, steady-state levels of isoprene were reached rapidly (i.e., within 15 to 30 minutes) after the onset or exposure.

There were substantial differences in the toxicokinetics of inhaled isoprene in mice compared to rats. In mice, fractional retention of inhaled isoprene, which reflects, in part, metabolism of isoprene, was linearly related to exposure concentrations up to 200 ppm but decreased at 2000 ppm; in rats, fractional retention of inhaled isoprene decreased with increasing exposure concentration over a range of exposures from 8 to 1500 ppm. Rats metabolized a greater fraction of the inhaled isoprene than did mice at all exposure concentrations. The differences in uptake and disposition between the two species should be considered in extrapolation of rodent data to humans.

29.07.2005 (4)

**Type** : Toxicokinetics

**Remark** : A physiological toxicokinetic (PT) model was developed for isoprene in mouse, rat and human. Experimentally determined partition coefficients were taken from the literature. Metabolic parameters were obtained from gas-uptake experiments. The measured data could be described by introducing hepatic and extrahepatic metabolism into the model. At exposure concentrations up to 50 ppm, the rate of metabolism at steady-state is 14 times faster in mice and about 8 times faster in rats than in humans. Isoprene accumulated only barely due to its fast metabolism and its low thermodynamic partition coefficient whole body:air. In addition, isoprene is produced endogenously. This production is negligible in rodents compared to that in humans (0.34 mmol /h/kg). About 90% of isoprene produced endogenously in humans is metabolized and 10% is exhaled unchanged. The blood concentration of isoprene in non-exposed humans is predicted to be 9.5 nmol/l. The area under the blood concentration-time curve (AUC) following exposure over 8 h to 10 ppm isoprene is about 4 times higher than the AUC resulting from the unavoidable endogenous isoprene over 24 h. A comparison of such AUCs can be used for establishing workplace exposure limits. For estimation of the absolute risk, knowledge of the body burden of the epoxide intermediates of isoprene is required. However, such data are not yet available.

29.07.2005 (11)

**Type** : Toxicokinetics

**Remark** : Male Fischer 344 rats exposed by nose-only inhalation for 6 hours to 8, 260, 1480, and 8200 ppm [4-14C] isoprene retained 19, 9, 6, and 5% of the inhaled radio-ac-ti-vity, respectively. About 75% of the retained isoprene radio-acti-vity was excreted in urine within 66 hours. Liver, blood, and especially fat contained the most isoprene and metabolites. During the in-hala-tion phase, respira--tory tract tissues contained concentrations of volatile metabolites substantial-ly out of proportion to their mass relative to liver and blood; this was inter-preted to indicate metabolism in the respiratory tract. Most of the radio-activity in blood (>85%) was associated with material of low volatility, probably mostly con-jugates or tetrols. Between 0.031% (at 8 ppm) and 0.002% (at 8200 ppm) of the inhaled 4-14C label was tentatively identified as isoprene diepoxide. Thus, the relative amount of the metabolites present in rat blood was highest for low con-cen-trations of inhaled isoprene. Under the assumption that all radioactive material with the volatility of the diepoxide was indeed the diepoxide, blood diepoxide con-cen-tra-tions of 0.37, 7.4, 15, and 17 mmol/L were derived from 6-hour exposures to 8, 260, 1480, and 8200 ppm, respectively.

29.07.2005 (12)

**Type** : Toxicokinetics

**Remark** : This study describes the in vitro biosynthesis of isoprene from DL-mevalonate in the cytosolic fraction of rat liver. The data presented support the hypothesis that breath isoprene is the result of cellular mevalonate metabolism and arises from the non-enzymatic decomposition of a C5 unit.

29.07.2005 (15)

**Type** : Toxicokinetics

**Remark** : In this study, the concentration of isoprene, the main hydrocarbon of human breath, was measured in the blood of humans and in the blood of five different animal species, ie., rat, rabbit, dog, ewe and cow. In human blood, the concentrations of isoprene were between 15 and 70 nmol/liter with a mean value of 37 + 25 nmol/liter. In animals, traces of isoprene were unambiguously detected by mass spectrometry in the blood of all species tested. However, the levels were always lower than 1 nmol/liter.

29.07.2005 (7)

**Type** : Toxicokinetics

**Remark** : This study was conducted to provide a better understanding of the mechanisms of reactions of the epoxides of butadiene and isoprene with biologically relevant nucleophiles. The reactivity of the mono-epoxide of butadiene, i.e., ethenyloxirane and the mono-epoxide of isoprene, i.e., 2-ethenyl-2-methyloxirane were compared with oxygen, nitrogen and sulfur nucleophiles. It was discovered that 2-ethenyl-2-methyloxirane unexpectedly suffers cleavage by nitrogen and sulfur nucleophiles preferentially at the "neo pentyl"position C-3. The resulting adducts were obtained as homogeneous compounds and fully characterized by spectroscopic analysis.

29.07.2005 (2)

**Type** : Toxicokinetics

**Remark** : The metabolism of isoprene was investigated with microsomes from cell lines expressing eight different human cytochrome P450 enzymes (i.e., CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, CYP3A4). CYP2E1 showed the highest rates of formation of the isoprene monoepoxides 3,4-epoxy-3-methyl-1-butene (EPOX-1) and 3,4-epoxy-2-methyl-1-butene (EPOX-11), followed by CYP2B6. CYP2E1 was the only enzyme showing detectable formation of the diepoxide of isoprene, 2-methyl-1,2:3,4-diepoxybutane. Both isoprene monoepoxides were oxidized by CYP2E1 to the diepoxide at similar enzymatic rates.

To investigate species differences with regard to the role of epoxide hydrolase in the metabolism of isoprene monoepoxides, the epoxidation of isoprene by human liver microsomes was compared to that of mouse and rat liver microsomes. The amounts of monoepoxides formed as a balance between epoxidation and hydrolysis was measured in incubations with and without the epoxide hydrolase inhibitor cyclohexene oxide. Inhibition of epoxide hydrolase resulted in similar rates of monoepoxide formation in mouse, rat and man. Without inhibitor, however, the total amount of monoepoxides present at the end of the incubation period was twice as high for mouse liver microsomes than for rat and even 15 times as high as for human liver microsomes. Thus, differences in epoxide hydrolase activity between species may be of crucial importance for the toxicity of isoprene in the various species.

29.07.2005 (3)

**Type** : other: Metabolism and Mutagenicity

**Remark** : This study evaluated the in vitro biotransformation of isoprene by hepatic subcellular fractions from four rodent species, i.e., mouse, rat, rabbit and hamster. Isoprene metabolism showed the same pattern in all species tested. In all cases, isoprene was metabolized to two mono-epoxides ( i.e., 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene) and one di-epoxide (i.e., 2-methyl-1,2,3,4-diepoxybutane). Among the epoxide metabolites of isoprene only the di-epoxide proved to be mutagenic in the Ames Salmonella mutagenicity assay in strain TA100. Of note, the isoprene di-epoxide was found to have a half-life and mutagenic and alkylating activities similar to those of the structurally related butadiene di-epoxide. The butadiene di-epoxide has been shown to be mutagenic, clastogenic and carcinogenic.

29.07.2005 (22)

**Type** : other: Pharmacokinetics

**Remark** : This study investigated the pharmacokinetics of isoprene in male B6C3F1 mice and male Wistar rats. In a series of experiments conducted with either 2 male Wistar rats or 5 male B6C3F1 mice, animals were exposed to different initial concentrations of gaseous isoprene, up to 4000 ppm in a closed exposure system. Time-dependent concentration decline in the atmosphere of the system was determined by gas chromatography. Similarly, exhalation and accumulation of endogenously produced isoprene was determined in untreated mice and rats. In both species, metabolism of isoprene shows saturation kinetics. Below atmospheric concentrations of 300 ppm in rats and in mice, the rate of metabolism is directly proportional to the concentration. The low accumulation of isoprene in the body at low atmospheric concentrations suggests transport limitation of the metabolism. Only small amounts of isoprene taken up are exhaled as unchanged substance (i.e., 15% in rats and 25% in mice). The half life of isoprene is 6.8 minutes in rats and 4.4 minutes in mice. At concentrations above above 300 ppm the rate of metabolism does not increase further in proportion to the atmospheric concentration. It finally approaches maximal values of 130 mmol / (h x kg) body weight atmospheric concentrations above 1500 ppm in rats, and 400 mmol / (h x g) body weight at concentrations above 2000 ppm in mice. This indicates limited production of the two possible mono-epoxides of isoprene at high concentrations.

Isoprene is endogenously produced and is systemically available. Its production rate is 1.9 mmol / (h x kg) in rats, and 0.4 mmol / (h x kg) in mice, respectively. Part of the endogenous isoprene is exhaled by the animals but it is metabolized to a greater extent: the rate of metabolism of endogenously produced and systemically available isoprene is 1.6 mmol / (h x kg) (rats) and 0.3 mmol / (h x kg) (mice).

29.07.2005 (58)

**Remark** : The purpose of this study was to obtain comparative pharmacokinetic data on the metabolism of inhaled isoprene in rats and mice. Inhalation studies were conducted in male Wistar rats and male B6C3F1 mice to investigate possible species differences in metabolism of this compound. In these studies two rats or five mice were placed in a closed 6.4 L dessicator jar chamber, equipped with 135 g soda lime for CO2 absorption and an oxygen supply. The animals were exposed to initial concentrations between about 5 ppm and 5000 ppm isoprene. Concentration changes of the compound in the gas phase of the system were measured by gas chromatography. Similarly, exhalation and accumulation of isoprene

endogenously produced by untreated animals while in the closed exposure system was determined. Kinetic parameters were determined from the concentration time-courses thus obtained, based on a two compartment pharmacokinetic model developed by Filser and Bolt.

For rats and mice, linear pharmacokinetics apply at exposure concentrations below 300 ppm isoprene. Saturation of isoprene metabolism is practically complete at atmospheric concentrations of about 1000 ppm in rats and about 2000 ppm in mice. In the lower concentration range where first-order metabolism applies, metabolic clearance of inhaled isoprene per kilogram body weight was 6200 mL/hr for rats and 12,000 mL/hr for mice. The estimated maximal metabolic elimination rates were 130 mmol /hr/kg for rats and 400 mmol /hr/kg for mice. This shows that the rate of isoprene metabolism in mice is about two or three times that in rats.

When the untreated animals are kept in a closed all-glass exposure system, the exhalation of isoprene into the system can be measured. This shows that the isoprene endogenously produced by the animals is systemically available within the animal organism. From such experiments the endogenous production rate of isoprene was calculated to be 1.9 mmol /hr/kg for rats and 0.4 mmol /hr/kg for mice. These data indicate that the endogenous production or isoprene should be accounted for when discussing a possible carcinogenic or mutagenic risk of this compound.

29.07.2005 (59)

**Remark** : The haemoglobin adduct formation measured after i. p. injection into male Sprague-Dawley rats and male B6C3F1 mice was linearly related to administered dose up to 500 µmol/kg and showed the same slope with both species. Dose correction for isoprene exhalation resulted in haemoglobin adduct formation of 0.16 and0.08 pmol Hb adduct/mg globin per µmol retained isoprene/kg body weight for mice and rats, respectively.

**Source** : Deutsche Shell Chemie GmbH Eschborn
Exxon Chemical Europe Inc. Bruxelles

29.07.2005 (72)

(1) Anderson D (2001). Genetic and reproductive toxicity of butadiene and isoprene, Chemico-Biological Interactions, Vol. 135-136, pp. 65-80.

(2) Bleasdale C, Small R, Watson W, Wilson J and Golding B (1996). Studies on the molecular toxicology of buta-1,3-diene and isoprene epoxides, Toxicology, Vol. 113, pp. 290-293.

(3) Bogaards J, Venekamp J and van Bladeren P (1996). The biotransformation of isoprene and the two isoprene monoepoxides by human cytochrome P450 enzymes, compared to mouse and rat liver microsomes, Chemico-Biological Interactions, Vol. 102, pp. 169-182.

(4) Bond J, Bechthold W, Birnbaum L, Dahl A, Medinsky M, Sun J, and Henderson R (1991). Disposition of inhaled isoprene in B6C3F1 mice, Toxical. Appl. Pharmacol., Vol. 107, pp. 494-503.

(5) Budavari S (ed.) (1996). The Merck Index - an encyclopedia of chemicals, drugs, and biologicals, Twelfth edition. Merck & Co., Inc., Whitehouse Station, NJ, USA.

(6) Cailleux A and Allain P (1989). Isoprene and sleep, Life Sciences, Vol. 44, pp. 1877-1880.

(7) Cailleux A, Cogny M and Allain P (1992). Blood isoprene concentrations in humans and in some animal species, Biochemical Medicine and Metabolic Biology, Vol. 47, pp. 157-160.

(8) Chiou C, Freed V, Schmedding D and Kohnert R (1977). Partition coefficient and bioaccumulation of selected organic chemicals, Environ. Sci. Technol., Vol. 11, No. (5), pp. 475-478.

(9) CITI (Chemicals Inspection & Testing Institute) (1992). Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. Japan Chemical Industry Ecology-Toxicology & Information Center.

(10) Cleveland C and Yavitt J (1998). Microbial consumption of atmospheric isoprene in a temperate forest soil, Applied Environ. Microbiol., Vol 64, pp. 172-177.

(11) Csanady G and Filser J (2001). Toxicokinetics of inhaled and endogenous isoprene in mice, rats, and humans, Chemico-Biological Interactions, Vol. 135-136, pp. 679-685.

(12) Dahl A, Birnbaum L, Bond J, Gervasi P and Henderson R (1987). The fate of isoprene inhaled by rats: Comparison to Butadiene, Toxicology and Applied Pharmacology, Vol. 89, pp. 237-248.

(13) De Meester C, Mercier M and Poncelet F (1981). Mutagenic activity of butadiene, hexachlorobutadiene and isoprene. In: Industrial and Environmental Xenobiotics. Edited by I Gut, M Cirkt and GL Plaa. Springer Verlag, Berlin, pp. 195-203.

(14) Del Monte M, Citti L and Gervasi P (1985). Isoprene metabolism by liver microsomal monooxygenases, Xenobiotica, Vol. 15, pp. 591-597.

(15) Deneris E, Stein R and Mead J (1984). In vitro biosynthesis of isoprene from mevalonate utilizing a rat liver cytosolic fraction, Biochem Biophy Res Comm., Vol. 123, No. 2, pp. 691-696.

(16) Derwent R, Jenkin M and Saunders S (1996). Photochemical ozone creation potentials for a large number of reactive hydrocarbons under European conditions, Atmospheric Environ., Vol. 30, pp. 181-199.

(17) Derwent R, Jenkin M, Saunders S and Pilling M (1998). Photochemical ozone creation potentials for organic compounds in Northwest Europe calculated with a master chemical mechanism, Atmospheric Environ., Vol. 32, pp. 2429-2441.

(18) EPIWIN (1999). Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.

(19) EPIWIN (2000). Estimation Program Interface for Windows, version 3.11. Syracuse Research Corporation, Syracuse, NY, USA.

(20) ExxonMobil Biomedical Sciences, Inc. (2004). Ready Biodegradability, Manometric Respirometry. Study #177294A.

(21) Galloway S, Armstrong M, Reuben C, Colman S, Brown B, Cannon C, Bloom A, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin B, Resnick M, Anderson B and Zeiger E (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals, Environ Mol. Mutagen, Vol. 10, pp. 1-175.

(22) Gervasi P and Longo V (1990). Metabolism and mutagenicity of isoprene, Environmental Health Persepctives, Vol. 86, pp. 85-87.

(23) Gervasi P, Citti L, Del Monte M, Longo V and Benetti D (1985). Mutagenicity and chemical reactivity of epoxidic intermediates of the isoprene metabolism and other structurally related compounds, Mutat. Res., Vol. 156, pp. 77-82.

(24) Gostinskii V (1965). Toxicity of isoprene and maximal safe concentration of the vapour in the air, Fed. Proc. (Transl. Suppl.), Vol. 24, pp. 1123-1126.

(25) Gould E (1959). Mechanism and Structure in Organic Chemistry. Holt, Reinhart and Winston, New York, NY, USA.

(26) Green MHL (1984). Mutagen testing using trp+ reversion in Escherichia coli. In: Handbook of Mutagenicity Test Procedures. Kilbey BJ, Legator M, Nichols W and Ramel C (Eds.). 2nd edition, pp.161-187. Elsevier Science Publishers BV, Amsterdam.

(27) Hansch C and Leo A (1985). MedChem Project Issue No. 26. Pomona College, Claremont, CA, USA, (cited in: CIS Envirofate, 1992, Online database).

(28) Hanst P, Spence J and Edney E (1980). Carbon monoxide production in photooxidation of organic molecules in the air, Atmospheric Environ., Vol. 14, No. 9, pp. 1077-1088.

(29) Harris J (1982). Rate of Hydrolysis. In: Handbook of Chemical Property Estimation Methods. Chapter 7. Edited by WJ Lyman, WF Reehl and DH Rosenblatt. McGraw-Hill Book Company, New York, NY, USA.

(30) Hawley G (1981). The Condensed Chemical Dictionary. 10th Ed. Van Nostrand Reinhold Company Inc. New York, NY, USA.

(31) Howard P, Boethling R, Jarvis W, Meylan W, and Michalenko E (1991). Handbook of Environmental Degradation Rates. Lewis Publishers, Inc. Chelsea, MI, USA, pp. 176-177.

(32) Huntingdon Life Sciences (UK) (2003). Isoprene - Bacterial Reverse Mutation Tests Incorporating Mouse S9 and Microsome Fractions. Final Report. Conducted at Huntingdon Life Sciences (UK); sponsor - International Institute of Synthetic Rubber Producers. December 2003.

(33) Huntingdon Life Sciences Ltd. (2003a). Assessment of Biodegradability Using the Closed Bottle Method. Project ID CSS/036. Huntingdon Life Sciences Ltd., Cambridgeshire, England.

(34) Huntingdon Life Sciences Ltd. (2003b). Acute Toxicity to Rainbow Trout (Semi-static exposure conditions). Project ID CSS 032. Huntingdon Life Sciences Ltd., Cambridgeshire, England.

(35) Huntingdon Life Sciences Ltd. (2003c). Acute Toxicity to Daphnia Magna. Project ID CSS 033. Huntingdon Life Sciences Ltd., Cambridgeshire, England.

(36) Huntingdon Life Sciences Ltd. (2003d). Algal Growth Inhibition Assay. Project ID CSS 029. Huntingdon Life Sciences Ltd., Cambridgeshire, England.

(37) Keeler P, Yokel H and Vaughn C (1976). Toxicological properties of an isoprene process stream. The Dow Chemical Company, Midland, MI, USA. (Unpublished report). (Quoted in: Workplace Environmental Exposure Level Guide Isoprene (1990). American Industrial Hygiene Association.)

(38) Kimmerle G and Solmecke B (1972). Isopren-Akute Toxizit, tsuntersuchungen. Bayer AG, unveroffentlichter Bericht Nr. 3373.

(39) Kushi A, Yoshida D and Mizusaki S (1985). Mutagenicity of gaseous nitrogen oxides and olefins on Salmonella TA 102 and TA 104, Mutat. Res., Vol. 147, pp. 263-264 (conference abstract).

(40) Lacson J, Kaelin T and Yoneyama M (2005). Isoprene. SRI Abstract CEH. http://www.sriconsulting.com/CEH/Public/Reports/446.0000/

(41) Longo V, Citti L and Gervasi P (1985). Hepatic microsomal metabolism of isoprene in various rodents, Toxicol. Lett., Vol. 29, pp. 33-37.

(42) Mackay D (1998). Level I Fugacity-Based Environmental Equilibrium Partitioning Model, Version 2.1 (16-bit). Environmental Modelling Centre, Trent University, Ontario, Canada.

(43) Mackay D (1998). Level III Fugacity-Based Environmental Equilibrium Partitioning Model, Version 2.1 (16-bit). Environmental Modelling Centre, Trent University, Ontario, Canada.

(44) Mamedov A (1979). Response of lymphoid tissue to single and multiple inhalation exposures to isoprene and some relevant integral indices, AM Gigiena Truda I Professional'Nye Zabolevaniya, Vol. 6, pp. 34-37.

(45) Mast T, Evanoff J, Stoney K, Westerberg R and Rommereim R (1989). Inhalation developmental toxicology studies: teratology study of isoprene in mice and rats. Final report. Govt. Rep. Announce. Index 14.

(46) Mast T, Rommereim R, Weigel R, Stoney K, Schwetz B and Morrissey R (1990). Inhalation developmental toxicity of isoprene in mice and rats, Toxicologist, Vol. 19, pp. 42.

(47) McAuliffe C (1966). Solubility in water of Paraffin, Cycloparaffin, Olefin, Acetylene, Cycloolefin, and Aromatic Hydrocarbons. J. Physical Chem. 70, 1267-1275.

(48) Melnick R, Roycroft J, Chou B, Ragan H and Miller R (1990). Inhalation toxicology of isoprene in F344 and B6C3F1 mice following two-week exposures, Environ. Health Perspect., Vol. 86, pp. 93-98.

(49) Melnick R, Sills R, Roycroft J, Chou B, Ragan H and Miller R (1994). Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure, Cancer Res., Vol. 54, pp. 5333-5339.

(50) Mitin Y (1969). Changes in the upper respiratory tract in isoprene rubber production workers,. Zh. Ushn. Nos. Gorl. Bolezn. Vol. 29, pp. 79-83. (Abstract in English)

(51) 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, Environ. Mutagen., Vol. 8 (Suppl. 7), pp. 1-119.

(52) National Toxicology Program (1983). Salmonella Mutagenesis Test Results. NTP Tech. Bull. 9, 5-6.

(53) National Toxicology Program (1989). Inhalation Developmental Toxicology Studies: Teratology Study of Isoprene in Mice and Rats. TER88045; NTIS#DE89008095.

(54) National Toxicology Program (1999). Toxicology and Carcinogenesis Studies of Isoprene (CAS No. 78-79-5) in F344/N Rats (Inhalation Studies). NTP TR-486. NIH Publication No. 99-3976.

(55) National Toxicology Program. Toxicity Studies of Isoprene (CAS No. 78-79-5), Administration by Inhalation to F344/N Rats and B6C3F1 Mice. United States Department of Health and Human services, Public Health Service, National Institutes of Health. NTP Technical Report No. 31 (in press).

(56) Niki H, Maker P, Savage C and Breikenbach L (1983). Atmospheric ozone-olefin reactions, Environ. Sci. Toxicol., Vol. 17, pp. 312A-322A.

(57) O'Neil MJ, Smith A, Heckelman PE and Budavari S (eds.) (2001). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Thirteenth Edition. Merck Research Laboratories, Merck & Co., Inc. Whitehouse Station, NJ, USA.

(58) Peter H, Wiegand H, Bolt H, Greim H, Walter G, Berg M and Filser J (1987). Pharmacokinetics of isoprene in mice and rats, Toxicol. Lett., Vol. 36, pp. 9-14.

(59) Peter H, Wiegand H, Filser J, Bolt H and Laib R (1990). Inhalation pharmacokinetics of isoprene in rats and mice, Environmental Health Perspectives, Vol. 86, pp. 89-92.

(60) Pickering Q and Henderson C (1966). Acute toxicity of some important petrochemicals to fish, J. Water Pollut. Cont. Fed., Vol. 38, No. 9, pp. 1419-1429.

(61) Placke M, Griffis L, Bird M, Bus J, Persing R and Cox L Jr (1996). Chronic inhalation oncogenicity study of isoprene in B6C3F1 mice, Toxicology, Vol. 113, pp. 253-262.

(62) Placke M, Persing R, Cox T, Griffis L, Bus J and Bird M. Inhalation oncogenicity study of isoprene in B6C3F1 mice. (Study not yet published.)

(63) Sandmeyer E (1981). Aliphatic hydrocarbons. In: Patty's Industrial Hygiene and Toxicology (1981). Edited by DG Clayton and FE Clayton. Wiley, New York, NY, USA, pp. 3208-3220.

(64) Shamberger R (1971). Inhibitory effect of vitamin A on carcinogenesis, J. Natl. Cancer Inst., Vol. 47, pp. 667-673.

(65) Shelby M (1990). Results of NTP-sponsored mouse cytogenetic studies on 1,3-butadiene, isoprene and chloroprene, Environ. Health Perspect., Vol. 86, pp. 71-73.

(66) Shell Research Group Report (1984). Report # SGBR.84.032, Sittingbourne Research Centre, Sittingbourne, Kent, England.

(67) Shell Research Group Report (1984). Report # SGBR.84.090, Sittingbourne Research Centre, Sittingbourne, Kent, England.

(68) Shugaev B (1969). Concentrations of hydrocarbons in tissues as a measure of toxicity, Arch. Environ. Health, Vol. 18, pp. 878-882.

(69) Shugaev B (1969). Concentrations of hydrocarbons in tissues as a measure of toxicity,. Arch. Environ. Health, Vol. 18, pp. 878-882.

(70) Small R, Golding B and Watson W (1997). Species differences in the stereochemistry of the metabolism of isoprene in vitro, Xenobiotica, Vol. 2, pp. 1155-1164.

(71) SRI International (2000). SRI Consulting, Menlo Park, CA, USA.

(72) Sun J, Dahl A, Bond J, Birnbaum L and Henderson R (1989). Characterization of haemoglobin adduct formation in mice and rats after administration of 14C butadiene or 14C isoprene,. Toxicol. Appl. Pharmacol., Vol. 100, pp. 86-95.

(73) Tice R, Boucher R, Luke C, Paquette D, Melnick R and Shelby M (1988). Chloroprene and isoprene: cytogenetic studies in mice. Mutagenesis 3 (2), 141-146.

(74) Tsutsumi S, Yamaguchi T, Komatsu S and Tamura S (1969). On the teratogenic effects of vitamin A-like substances. Proc. Congenital Anomalies Res. Assoc., Annual Report No. 9, 27.

(75) USITC (United States International Trade Commission) (1995). Washington, DC, USA.

(76) Watson W, Cottrell L, Zhang D and Golding B (2001). Metabolsim and molecular toxicology of isoprene, Chemico-Biological Interactions, Vol. 135-136, pp. 223-238.

(77) Wistuba D, Weigand K and Peter H (1994). Stereoselectivity of in vitro isoprene metabolism,. Chem Res Toxicol., Vol. 7, No. 336-343.

(78) Zepp R and Cline D (1977). Rates of direct photolysis in the aqueous environment, Environ. Sci. Technol., Vol. 11, pp. 359-366.

(79) Zwolinski BJ and Wilhoit RC (1971) Handbook of Vapor Pressures and Heats of Vaporization of Hydrocarbons and Related Compounds. API44-TRC101. Thermodynamics Research Center, College Station, TX, USA.

European Food Safety Authority

EFSA Journal 2015;13(4):4069

## SCIENTIFIC OPINION

# Scientific opinion on Flavouring Group Evaluation 25, Revision 3 (FGE.25Rev3): Aliphatic hydrocarbons from chemical group 31[1]

**EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]**

European Food Safety Authority (EFSA), Parma, Italy

## ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 14 flavouring substances in the Flavouring Group Evaluation 25, Revision 3, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances was considered to have a genotoxic potential. This revision is made due to the inclusion of new toxicity data on the supporting substances β-caryophyllene [FL-no: 01.007] and myrcene [FL-no: 01.008] considered in FGE.78Rev2 to cover the assessment of 4(10)-thujene [FL-no: 01.059], 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070]. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that all 14 substances [FL-no: 01.001, 01.027, 01.028, 01.033, 01.034, 01.035, 01.038, 01.039, 01.046, 01.054, 01.057, 01.059, 01.064, 01.070] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. Adequate specifications including complete purity and identity criteria for the materials of commerce have been provided for all 14 candidate substances.



## KEY WORDS

flavourings, aliphatic, hydrocarbons, FGE.25.

[1] On request from the European Commission, Question No EFSA-Q-2013-00193, EFSA-Q-2013-00849, EFSA-Q-2013-00850 and EFSA-Q-2013-00851 adopted on 18 March 2015.

[2] Panel members: Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Konrad Grob, Rainer Gürtler, Trine Husøy, Wim Mennes, Maria Rosaria Milana, André Penninks, Maria de Fatima Tavares Poças, Vittorio Silano, Andrew Smith, Christina Tlustos, Fidel Toldra, Detlef Wölfle and Holger Zorn. Correspondence: fip@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings: Ulla Beckman Sundh, Leon Brimer, Karl-Heinz Engel, Rainer Gürtler, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft and Karin Nørby and EFSA staff: Annamaria Rossi, Maria Carfi and Maria Anastassiadou for the support provided to this scientific opinion.

Suggested citation: EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific Opinion on Flavouring Group Evaluation 25, Revision 3 (FGE.25Rev3): Aliphatic hydrocarbons from chemical group 31. EFSA Journal 2015;13(4):4069, 115 pp. doi:10.2903/j.efsa.2015.4069

Available online: www.efsa.europa.eu/efsajournal

## SUMMARY

Following a request from the European Commission, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 14 flavouring substances in the Flavouring Group Evaluation 25, Revision 3 (FGE.25Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 14 flavouring substances belong to chemical group 31, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.25, Revision 3 includes the assessment of a 90-day study on ß-caryophyllene [FL-no: 01.007] supporting the candidate substance 4(10)-thujene [FL-no: 01.059] and a 90-day study on myrcene [FL-no: 01.008] supporting the candidate substances 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070].

Since the publication of FGE.25Rev2, Industry has informed EFSA that 23 substances [former FL-no: 01.021, 01.022, 01.023, 01.030, 01.031, 01.032, 01.036, 01.037, 01.042, 01.043, 01.044, 01.047, 01.050, 01.051, 01.052, 01.053, 01.055, 01.056, 01.058, 01.060, 01.066, 01.067 and 01.078] are no longer supported for use as flavouring substances in Europe by Industry. The FGE.25Rev3 therefore deals with 14 flavouring substances in total.

The 14 candidate substances are aliphatic hydrocarbons from chemical group 31, which have been divided into four subgroups: I) acyclic alkanes, II) acyclic alkenes, III) cyclohexene hydrocarbons, IV) is now empty as all the substances are no longer supported by Industry, V) bicyclic, non-aromatic hydrocarbon ,VI) is now empty as all the substances are no longer supported by Industry.

Seven of the 14 flavouring substances possess chiral centres and three can exist as geometrical isomers.

All of the 14 candidate substances are classified into structural class I, according to the decision tree approach presented by Cramer et al. (1978).

Twelve of the 14 candidate substances have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the “Maximised Survey-derived Daily Intake” (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel decided also to perform an estimate of the daily intakes per person using a “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, 12 of the 14 flavouring substances in this group have intakes in Europe from 0.0085 to 14 µg/*capita* per day, which are below the threshold of concern value for structural class I (1800 µg/person per day) substances. For limonene [FL-no: 01.001] and l-limonene

[FL-no: 01.046] the intakes are 4000 and 2100 µg/*capita* per day, which are above the threshold of concern value for structural class I (1800 µg/person per day).

On the basis of the reported annual production volumes in Europe (MSDI approach), the total combined intakes of the candidate and supporting substances can be calculated for the substances in subgroup I, II, III and V (those subgroups still containing substances) evaluated through the Procedure.

Subgroup I (acyclic alkanes): the combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Appendix A), is 3.0 µg/*capita* per day, which does not exceed the threshold of 1800 µg/person per day.

Subgroup II (acyclic alkenes): the combined intake of the three candidate substances, all from structural class I and evaluated via the B-side of the Procedure (Appendix A), is 23 µg/*capita* per day, which does not exceed the threshold of 1800 µg/person per day.

Subgroup III (cyclohexene hydrocarbons): the combined intake of the three candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Appendix A), is 6100 µg/*capita* per day, which does exceed the threshold of 1800 µg/person per day. The total combined intake of the three candidate and four supporting substances (also from structural class I) is approximately 42 000 µg/*capita* per day. This intake exceeds the threshold of 1800 µg/person per day for a structural class I substance. However, together, limonene [FL-no: 01.001], *l*-limonene [FL-no: 01.046] and *d*-limonene (supporting substance [FL-no: 01.045]) account for approximately 40 000 µg/*capita* per day. The total combined intake of 42 000 µg/*capita* per day for the candidate and the supporting substances corresponds to 700 µg/kg bw per day for a person with a body weight of 60 kg. Thus, based on the NOAEL for *d*-limonene of 215 mg/kg bw per day, a margin of safety of 307 can be calculated, and accordingly these substances are not expected to be of safety concern at the estimated level of intake.

Subgroup V (bicyclic, non-aromatic hydrocarbons): as no sufficient data are available to conclude that the candidate substance [FL-no: 01.059] will be metabolised to innocuous products, it is evaluated via the B-side of the Procedure (Appendix A). The total combined intake of the one candidate and eight supporting substances (also from structural class I) is approximately 3800 µg/*capita* per day. This intake exceeds the threshold of 1800 µg/person per day for a structural class I substance. However, three supporting substances, pin-2(10)-ene [FL-no: 01.003], pin-2(3)-ene [FL-no: 01.004] and β-caryophyllene [FL-no: 01.007] together account for approximately 3400 µg/*capita* per day. The total combined intake of 3800 µg/*capita* per day for the candidate and the supporting substances corresponds to 63 µg/kg bw per day for a person with a body weight of 60 kg. Thus, based on the NOAEL for β-caryophyllene of 222 mg/kg bw per day, a margin of safety of 3500 can be calculated, and accordingly this substance is not expected to be of safety concern at the estimated level of intake.

The available information on metabolism of the 14 candidate substances evaluated through the Procedure or the supporting substances for this FGE was limited. For the following 10 candidate substances it can be concluded that they will be metabolised into innocuous metabolites: [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] from subgroup I and [FL-no: 0.001, 01.027, 01.028, 01.039 and 01.046] from subgroup III. For two candidate substances there are data, which show that they may be metabolised to toxic metabolites [FL-no: 01.064 and 01.070]. For the remaining two candidate substances [FL-no: 01.035 and 01.059], the information is too limited and it cannot be assumed that they are metabolised to innocuous metabolites.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is concluded that the 10 candidate substances which are expected to be metabolised to innocuous substances, [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] from subgroup I and [FL-no: 01.001,

01.027, 01.028, 01.039 and 01.046] from subgroup III, would not give rise to safety concerns at their estimated intakes arising from their use as flavouring substances based on the MSDI approach.

For 4(10)-thujene [FL-no: 01.059], from subgroup V, which is not expected to be metabolised to innocuous substances, a margin of safety could be calculated based upon a NOAEL (222 mg/kg/bw) for the supporting substance ß-caryophyllene [FL-no: 01.007]. Compared to the MSDI of 4(10)-thujene of 14 µg/*capita* per day corresponding to 0.2 µg/kg bw per day, the NOAEL provides a margin of safety of $9.5 \times 10^5$.

For the three remaining substances, 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070] a margin of safety could be calculated based upon a NOAEL (44 mg/kg bw) for the supporting substance myrcene [FL-no: 01.008]. Compared to the MSDI of 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070] of 9.1, 14 and 0.0085 µg/*capita* per day equal to 0.15, 0.23 and 0.00014 µg/kg bw per day, the NOAEL provides a margin of safety of $2.9 \times 10^5$, $1.9 \times 10^5$ and $3.1 \times 10^8$.

The mTAMDI values for 11 candidate substances are above the threshold for structural class I of 1800 µg/person per day. For these substances more reliable exposure data are requested in order for them to be considered using the Procedure. For limonene [FL-no: 01.001] the mTAMDI is 1800 µg/person per day and for each of l-limonene and 1-octene [FL-no: 01.046 and 01.070] the mTAMDI is 1600 µg/person per day.

In order to determine whether this conclusion could be applied to the materials of commerce, it is necessary to consider the available specifications. The specifications including complete purity criteria and identity for the materials of commerce have been provided for all 14 flavouring substances.

Thus, for all 14 candidate substances [FL-no: 01.001, 01.027, 01.028, 01.033, 01.034, 01.035, 01.038, 01.039, 01.046, 01.054, 01.057, 01.059, 01.064 and 01.070] the Panel concluded that they would present no safety concern at their estimated levels of intake based on the MSDI approach.

## TABLE OF CONTENTS

## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings in food is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[4] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[5]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

### FGE.78Rev1

On 19 May 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 78, Revision 1 (FGE.78Rev1): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2[7].

The substances [FL-no: 01.008, 01.018, 01.040 and 01.061] were among the 14 substances for which the Panel had "reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or missing information on stereoisomerism/composition of mixture)" and also among those for which "additional toxicity data was requested".

### FGE.25Rev2

On 19 May 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): Aliphatic and aromatic hydrocarbons from chemical group 31[8].

The substances with [FL-no: 01.035, 01.064, 01.070 and 01.035] were among the 27 candidate substances for which "additional toxicity data" were required by EFSA. For [FL-no: 01.035] also "additional information on composition" was requested.

### FGE.18Rev2

On 30 September 2010, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8.

For the flavouring substance [FL-no: 02.146], the Panel considered that "additional data" are needed including "information on specifications/stereoisomerism/composition of mixture".

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p.34-50.

[5] Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC.OJ L 267, 2.10.2012, p. 1-161.

[6] Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8-16.

[7] EFSA Journal 2011;9(6):2178

[8] EFSA Journal 2011;9(6):2177

On 21 November 2012, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted a statement on the re-evaluation of 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] based on additional data on a supporting substance[9].

The Panel concluded that "linalool [FL-no: 02.013] is not sufficiently structurally related to 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] for a re-evaluation of [FL-no: 02.146]. Accordingly, "a 90-day study on 3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] or on a sufficiently structurally related substance has to be provided in order to establish on appropriate NOAEL".

**New data and relationship with other substances**

On 5 and 11 July 2013, the applicant submitted additional data on the following acyclic terpene hydrocarbons [FL-no: 01.008, 01.018, 01.040, 01.061, 01.035, 01.064, 01.070 and 02.146, represented by myrcene [FL-no: 01.008].

As regards the related substances also evaluated in these opinions, namely [FL-no: 01.003, 01.004, 01.007, 01.009, 01.017, 01.024, 01.026, 01.029 and 01.059], data was submitted and are currently being evaluated (EFSA-Q-2013-00185 to – 00193).

As regards substance with [FL-no: 01.014], data should be submitted by 31 December 2013[10].

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority (EFSA) to finalise its safety assessment on this group of flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

## SUPPORTING DOCUMENTS

Submission by the European Flavour Association

## INTERPRETATION OF THE TERMS OF REFERENCE

The above background and terms of reference include also a previous mandate received from the European Commission on 6 February 2013[11]. The present scientific opinion FGE.25Rev3 covers the safety assessment of the following flavouring substances: 4(10)-thujene with [FL-no: 01.059], 2,6-Dimethylocta-2,4,6-triene with [FL-no: 01.035], cis-3,7-Dimethyl-1,3,6-octatriene with [FL-no: 01.064] and 1-Octene with [FL-no: 01.070].

[9] EFSA Journal 2012;10(12):2995
[10] This substance is in the process of being deleted from the Union List (DG SANTE, 2015)
[11] SANCO.E3/SH/km D (2013) Ares(2013)15188

## ASSESSMENT

### 1. History of the Evaluation of the Substances in the Present FGE

The first version of the Flavouring Group Evaluation 25 (FGE.25) dealt with 32 aliphatic and aromatic hydrocarbons which have been divided into eight subgroups: I) acyclic alkanes, II) acyclic alkenes, III) cyclohexene hydrocarbons, IVa) benzene hydrocarbons, IVb) napthalene hydrocarbons, IVc) diphenylmethane, V) bi-and tricyclic, non-aromatic hydrocarbons and VI) macrocyclic, non-aromatic hydrocarbons. For one candidate substance, 2-methylbuta-1,3-diene [former FL-no: 01.049] (synonym: isoprene) evaluated in FGE.25 there was evidence of a genotoxic potential *in vivo* and of carcinogenic effects in experimental animals. Therefore, this substance could not be evaluated through the Procedure and could not be considered safe when used as a chemically defined flavouring substance. Subsequently, the substance has been deleted from the Register (Mennicke et al., 1983).

The first Revision of FGE.25 (FGE.25Rev1) included the assessment of three additional candidate substances [FL-no: 01.059, 01.070 and 01.078]. For two of these substances additional genotoxicity data on structurally related substances have been provided. A survey in open literature did not result in further data.

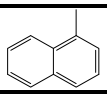
The second Revision of FGE.25 (FGE.25Rev2) included the assessment of three additional candidate substances [FL-no: 01.001, 01.021 and 01.046]. No toxicity or metabolism data were provided for these three substances. A survey in open literature did not result in further data for these three substances. In the FGE.25 and FGE.25Rev1, the Panel considered that additional toxicity data were needed for 26 of the substances evaluated through the Procedure as no adequate toxicity study from which a NOAEL could be established was available, neither on the candidate substances nor on supporting substances. Additional toxicity and genotoxicity data had become available for the supporting substance myrcene [FL-no: 01.008] as had additional genotoxicity data for one of the 26 substances [FL-no: 01.047] and on cedrene washed (unspecified cedrene). Since the publication of FGE.25Rev1 new tonnage data for [FL-no: 01.035, 01.047 and 01.064] had become available (Flavour Industry, 2010), and included in this revision. Industry has also submitted additional information on stereoisomeric composition [FL-no: 01.027, 01.032, 01.034, 01.035, 01.050, 01.055, 01.056 and 01.060], composition of mixture [FL-no: 01.078] and missing ID-test [FL-no: 01.078].

Since the publication of FGE.25Rev2, 23 substances [FL-no: 01.021, 01.022, 01.023, 01.030, 01.031, 01.032, 01.036, 01.037, 01.042, 01.043, 01.044, 01.047, 01.050, 01.051, 01.052, 01.053, 01.055, 01.056, 01.058, 01.060, 01.066, 01.067 and 01.078] are no longer supported for use as flavouring substances in Europe by Industry and will therefore not be considered any further (DG SANCO, 2012; DG SANCO, 2013). Information from the previous version of FGE.25 on these substances is collected in Appendix D. However, information on these substances will be kept in the main text if relevant for the remaining candidate substances. The 23 substances are listed here below.

| FL-no | EU Register name |
|---|---|
| 01.021 | delta-Cadinene |
| 01.022 | α-Cedrene |
| 01.023 | 1(5),11-Guaiadiene |
| 01.030 | β-Cubebene |
| 01.031 | 1,2-Dihydro-1,1,6-trimethylnaphthalene |
| 01.032 | 2,3-Dihydrofarnesene |
| 01.036 | Diphenylmethane |
| 01.037 | Dodec-1-ene |
| 01.042 | Germacra-1(10),4(14),5-triene |
| 01.043 | 3,7,10-Humulatriene |
| 01.044 | Isolongifolene |
| 01.047 | Longifolene |
| 01.050 | 3-Methylhexane |
| 01.051 | 2-Methylnaphthalene |

| FL-no | EU Register name |
|---|---|
| 01.052 | α-Muurolene |
| 01.053 | Naphthalene |
| 01.055 | β-Phellandrene |
| 01.056 | α-Santalene |
| 01.058 | 1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene |
| 01.060 | 1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane |
| 01.066 | 2-Cedrene |
| 01.067 | 8(14)-Cedrene |
| 01.078 | 2,4-Nonadiene |

As a consequence the following supporting substance has been deleted from this revision; moreover 1-methylnaphthalene is also in the process of being deleted from the Union List (DG SANTE, 2015)

| FL-no JECFA no | EU Register name | Structural formula |
|---|---|---|
| 01.014 1335 | 1-Methylnaphthalene |  |

The table below gives information on publication dates and links to the published versions.

| FGE | Opinion Adopted by EFSA | Link | No. of Candidate Substances |
|---|---|---|---|
| FGE.25 | 1 April 2008 | http://www.efsa.europa.eu/en/efsajournal/doc/918.pdf | 32 |
| FGE.25Rev1 | 23 September 2009 | http://www.efsa.europa.eu/en/scdocs/scdoc/1334.htm | 34 |
| FGE.25Rev2 | 18 May 2011 | http://www.efsa.europa.eu/en/efsajournal/pub/2177.htm | 37 |
| FGE.25Rev3 | 18 March 2015 | | 14 |

The present Revision of FGE.25, FGE.25Rev3, deals with additional toxicity data from a 90-day study provided for β-caryophyllene [FL-no: 01.007] considered in FGE.78Rev2 (EFSA, in press). This information is used for the assessment of 4(10)-thujene [FL-no: 01.059]. Furthermore, new short term study and genotoxicity data have been provided for [FL-no: 01.007] (EFFA, 2012). Additional toxicity data from a 90-day study provided for the supporting substance myrcene [FL-no: 01.008] are also evaluated. This information is used for the assessment of 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070]. Furthermore, new information on European production figures has been provided for [FL-no: 01.035, 01.064 and 01.070] (IOFI, 2013) and use levels have been provided for [FL-no: 01.001, 01.046 and 01.070] (EFFA, 2015).

## 2. Presentation of the Substances in Flavouring Group Evaluation 25, Revision 3

### 2.1. Description

The present Flavouring Group Evaluation 25, Revision 3 (FGE.25Rev3) using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000) (The Procedure – shown in schematic form in Appendix A of this FGE), deals with 14 aliphatic hydrocarbons (candidate substances) from chemical group 31, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000). The candidate substances in the group have been divided into the following subgroups:

I) Acyclic alkanes,

II) Acyclic alkenes,

III) Cyclohexene hydrocarbons,

IV) The group was divided into IVa) benzene hydrocarbons, IVb) naphthalene hydrocarbons, IVc) diphenylmethane; the substances previously allocated to these groups are no longer supported for use as flavouring substances in Europe,

V) Bicyclic, non-aromatic hydrocarbons and

VI) Macrocyclic, non-aromatic hydrocarbons. The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.

One flavouring substance, 2-methylbuta-1,3-diene [former FL-no: 01.049] (synonym: isoprene) evaluated in FGE.25 has been deleted from the Register of flavouring substances as it showed genotoxic potential *in vivo* and carcinogenic effects in experimental animals. Therefore this substance will not be further discussed as a candidate substance in the current Revision 3 of FGE.25 (FGE.25Rev3).

The 14 candidate substances under consideration, with their chemical Register names, FLAVIS -(FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

A summary of the safety evaluation of the candidate substances under consideration in the present evaluation are listed in Table 7.

The 14 candidate substances are closely related structurally to 18 flavouring substances (supporting substances) evaluated at the 63rd JECFA meeting (JECFA, 2005b) in the groups of "Aliphatic and alicyclic hydrocarbons". The supporting substances are listed in Table 8.

## SUMMARY OF SPECIFICATION DATA

**Table 1:** Specification Summary of the Substances in the Flavouring Group Evaluation 25, Revision 3

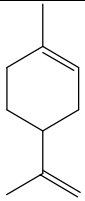
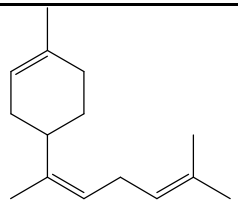
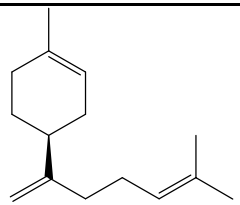
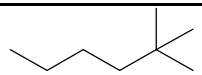
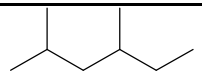
| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight | Solubility [a]<br>Solubility in ethanol [b] | Boiling point, °C [c]<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index [d]<br>Spec.gravity [e] | Specification comments |
|---|---|---|---|---|---|---|---|---|
| 01.001 | Limonene |  | 2633<br>491<br>138-86-3 | Liquid<br>$C_{10}H_{16}$<br>136.23 | Insoluble<br>Soluble | 178<br><br>MS<br>95 % | 1.4760-1.4820<br>0.843-0.851 | With respect to specific gravity it is noted that limonene and l-limonene are submitted by different applicants. |
| 01.027 | Bisabola-1,8,12-triene |  | 17627-44-0 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 99 (5 hPa)<br><br>MS<br>95 % | 1.483-1.489<br>0.912-0.918 | Racemate, mixture of (E)- and (Z)-isomers (EFFA, 2010). 50 - 70 % (E)-isomer (EFFA, 2013). CASrn in Register refers to the racemate. |
| 01.028 | beta-Bisabolene |  | 495-61-4 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 130 (13 hPa)<br><br>MS<br>95 % | 1.489-1.495<br>0.879-0.885 | CASrn in Register refers to the (4S)-isomer. |
| 01.033 | 2,2-Dimethylhexane |  | 590-73-8 | Liquid<br>$C_8H_{18}$<br>114.23 | Practically insoluble or insoluble<br>Freely soluble | 107<br><br>MS<br>95 % | 1.390-1.396<br>0.693-0.699 | |
| 01.034 | 2,4-Dimethylhexane |  | 589-43-5 | Liquid<br>$C_8H_{18}$<br>114.23 | Practically insoluble or insoluble<br>Freely soluble | 109<br><br>MS<br>95 % | 1.390-1.396<br>0.697-0.703 | Racemate (EFFA, 2010). |

**Table 1:** Specification Summary of the Substances in the Flavouring Group Evaluation 25, Revision 3

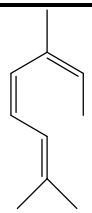
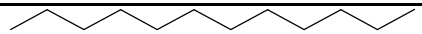
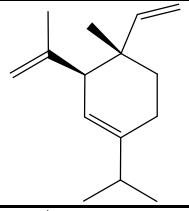
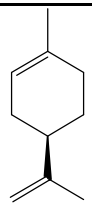
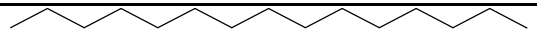
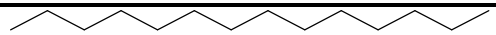
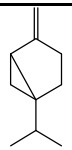
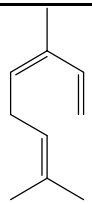
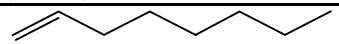
| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight | Solubility [a]<br>Solubility in ethanol [b] | Boiling point, °C [c]<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index [d]<br>Spec.gravity [e] | Specification comments |
|---|---|---|---|---|---|---|---|---|
| 01.035 | 2,6-Dimethylocta-2,4,6-triene |  | 673-84-7 | Liquid<br>$C_{10}H_{16}$<br>136.24 | Practically insoluble or insoluble<br>Freely soluble | 75 (13 hPa)<br><br>MS<br>95 % | 1.539-1.545<br>0.809-0.815 | Mixture of (E)- and (Z)-isomers (EFFA, 2010). 4E,6E (25 - 50 %); 4E,6Z (25 - 30 %); 4Z,6E (25 - 30 %); 4Z,6Z (10 - 20 %) (EFFA, 2013). CASrn in Register does not specify stereoisomeric composition. |
| 01.038 | Dodecane |  | 112-40-3 | Liquid<br>$C_{12}H_{26}$<br>170.34 | Practically insoluble or insoluble<br>Freely soluble | 216<br><br>MS<br>95 % | 1.417-1.423<br>0.746-0.752 | |
| 01.039 | delta-Elemene |  | 10996<br>20307-84-0 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 107 (13 hPa)<br><br>MS<br>95 % | 1.480-1.486<br>0.856-0.862 | CASrn in Register refers to the (3R, 4R)-isomer. |
| 01.046 | l-Limonene |  | 2633<br>491<br>5989-54-8 | Liquid<br>$C_{10}H_{16}$<br>136.23 | Insoluble<br>Soluble | 177<br><br>MS<br>95 % | 1.469 - 1.473<br>0.837 - 0.841 | With respect to specific gravity it is noted that limonene and l-limonene are submitted by different applicants. CASrn in Register refers to the (4S)-isomer. |
| 01.054 | Pentadecane |  | 629-62-9 | Liquid<br>$C_{15}H_{32}$<br>212.42 | Practically insoluble or insoluble<br>Freely soluble | 270<br>10<br>MS<br>95 % | 1.428-1.434<br>0.765-0.771 | |

**Table 1:** Specification Summary of the Substances in the Flavouring Group Evaluation 25, Revision 3

| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight | Solubility [(a)]<br>Solubility in ethanol [(b)] | Boiling point, °C [(c)]<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index [(d)]<br>Spec.gravity [(e)] | Specification comments |
|---|---|---|---|---|---|---|---|---|
| 01.057 | Tetradecane |  | <br><br>629-59-4 | Liquid<br>$C_{14}H_{30}$<br>198.39 | Practically insoluble or insoluble<br>Freely soluble | 252<br>5<br>MS<br>95 % | 1.422-1.428<br>0.759-0.765 | |
| 01.059 | 4(10)-Thujene |  | <br>11018<br>3387-41-5 | Liquid<br>$C_{10}H_{16}$<br>136.24 | Practically insoluble or insoluble<br>Freely soluble | 165<br><br>MS<br>96 % | 1.463-1.469<br>0.840-0.846 | Assay value of 96%: mixture of 70 % 4(10)-thujene, 6 % alpha-pinene, 19 % beta-pinene, 1 % myrcene, <4 % not identified (EFFA). |
| 01.064 | cis-3,7-Dimethyl-1,3,6-octatriene |  | <br><br>3338-55-4 | Liquid<br>$C_{10}H_{16}$<br>136.24 | Practically insoluble or insoluble<br>Freely soluble | 177<br><br>MS<br>95 % | 1.483-1.489<br>0.796-0.802 | Synonym: cis-beta-ocimene. |
| 01.070 | 1-Octene |  | 4293<br><br>111-66-0 | Liquid<br>$C_8H_{16}$<br>112.22 | Insoluble<br>Soluble | 121<br><br>IR NMR MS<br>97 % | 1.410-1.416<br>0.718-0.722 | |

(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1013.25 hPa, if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.

## 2.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.).

Seven of the 14 candidate substances possess chiral centres. For five of these chiral substances the chemical names and CAS numbers specify the stereoisomers. For two of the substances [FL-no: 01.027 and 01.034] information on the stereoisomeric composition has been provided (EFFA, 2010) (see Table 1).

Due to the presence and the position of double bonds three of the 14 candidate substances can exist as geometrical isomers. For two of these flavouring substances [FL-no: 01.027 and 01.035] Industry has provided the information on the ratios of these isomers (see Table 1). For [FL-no: 01.064] CAS number and name specify the configuration of the double bond.

## 2.3. Natural Occurrence in Food

Twelve of the 14 candidate substances have been reported to occur in various types of alcoholic beverages, chicken, egg (boiled), fish (raw), guinea hen, lamb fat, cheese, milk, butter, various herbs, various fruits and vegetables, tea, mace and liquorice.

**Table 2:** Candidate Substances Reported to Occur in Food (TNO, 2000; TNO, 2010)

| FL-no: | Name: | Quantitative data reported |
|---|---|---|
| 01.001 | Limonene | Up to 1.6 mg/kg in carrot, up to 0.3 in black currants, up to 1.4 mg/kg in tea and very high content in citrus oil |
| 01.028 | β-Bisabolene | 33.3 mg/kg in parsley, up to 1.18 mg/kg in carrot, 0.003 mg/kg in artichoke, 0.0003 mg/kg in guava fruit, trace amounts in mace and in nutmeg |
| 01.033 | 2,2-Dimethylhexane | Up to 0.9 mg/kg in tea |
| 01.034 | 2,4-Dimethylhexane | Up to 2 mg/kg in tea |
| 01.035 | 2,6-Dimethylocta-2,4,6-triene | 0.45 mg/kg in mango, 0.03 mg/kg in orange juice, up to 0.08 mg/kg in blackcurrants, trace amounts in sage |
| 01.038 | Dodecane | Up to 3.5 mg/kg in butter, up to 0.1 mg/kg in passiflora, 0.1 mg/kg in beans, 0.1 mg/kg in cocoa, 0.1 mg/kg in tea, up to 0.1 mg/kg in lamb, 0.08 mg/kg in dill, up to 0.05 mg/kg in strawberry, 0.02 mg/kg in chicken, up to 0.01 mg/kg in loquat, up to 0.01 mg/kg in papaya, 0.01 mg/kg in pea, up to 0.004 mg/kg in egg, 0.009 mg/kg in Guinea hen, 0.0006 mg/kg in raw fish, trace amounts in liquorice |
| 01.039 | delta-Elemene | Trace amounts in mandarin juice |

**Table 2:** Candidate Substances Reported to Occur in Food (TNO, 2000; TNO, 2010)

| FL-no: | Name: | Quantitative data reported |
|---|---|---|
| 01.054 | Pentadecane | Up to 2.3 mg/kg in butter, up to 0.4 mg/kg in mango, up to 0.346 mg/kg in cheese, 0.1 mg/kg in chicken, 0.1 mg/kg in tea, up to 0.05 mg/kg in strawberry, up to 0.03 mg/kg in raw fish, 0.029 mg/kg in Guinea hen, up to 0.02 mg/kg in egg (boiled), 0.014 mg/kg in milk powder, up to 0.01 mg/kg in papaya, 0.02 mg/kg in tamarind, 0.00001 mg/kg in aubergine, trace amounts in liquorice |
| 01.057 | Tetradecane | Up to 1.9 mg/kg in butter, 0.3 mg/kg in liquorice, up to 0.3 mg/kg in mango, up to 0.3 mg/kg in dill, 0.021 mg/kg in guinea hen, up to 0.15 mg/kg in cheese, 0.1 mg/kg in cocoa, 0.1 mg/kg in tea, 0.088 mg/kg in passiflora, up to 0.05 mg/kg in strawberry, up to 0.01 mg/kg in papaya, up to 0.003 mg/kg in egg, 0.001 mg/kg in milk 0.0005 mg/kg in chicken, 0.0008 mg/kg in raw fish, trace amounts in thymus, aubergine, coconut and lamb |
| 01.059 | 4(10)-Thujene | Up to 1000 mg/kg in caraway seed (oil), up to 1.9 mg/kg in blackcurrant, up to 5.2 mg/kg in carrot, up to 49000 mg/kg in cardamom (oil), up to 4000 mg/kg in coriander seed (oil), up to 4800 mg/kg in cumin seed (oil), 239000 mg/kg in pepper (oil) (different species), 334000 mg/kg in ginger (oil), up to 87600 mg/kg in laurel (oil), up to 510000 mg/kg in nutmeg (oil) |
| 01.064 | *cis*-3,7-Dimethyl-1,3,6-octatriene | Up to 13.6 mg/kg in guava fruit, up to 7.5 mg/kg in mango, 5.3 mg/kg in celery, 2.7 mg/kg in parsley, 2 mg/kg in dill, up to 0.6 mg/kg in tea, up to 0.5 mg/kg in papaya, up to 0.2 mg/kg in blackcurrant, 0.18 mg/kg in grapefruit, 0.05 mg/kg in cocoa, up to 0.01 mg/kg in passiflora, trace amounts in nectarine |
| 01.070 | 1-Octene | Up to 0.009 mg/kg in butter (1.7 mg/kg in heated butter), up to 0.001 mg/kg in boiled egg, 0.002 mg/kg in guinea hen |

According to TNO two of the substances have not been reported to occur naturally in any food items (Table 3):

**Table 3:** Candidate Substances Not Reported to Occur in Food (TNO, 2000; TNO, 2011)

| FL-no: | Name: |
|---|---|
| 01.027 | Bisabola-1,8,12-triene |
| 01.046 | *l*-limonene |

## 3. Specifications

Purity criteria for the 14 substances have been provided by the Flavour Industry (EFFA, 2005a; EFFA, 2006a; EFFA, 2006b; Flavour Industry, 2006; Flavour Industry, 2009) (see Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), this information is adequate for all 14 candidate substances (see Section 2.2 and Table 1).

## 4. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

### 4.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry (IOFI), in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[12] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present FGE.25Rev3 the total annual production volume of the 14 candidate substances from use as flavouring substances in Europe was reported to be approximately 51 000 kg[13] (EFFA, 2005a;

[12] EU figure 375 million. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

[13] The substances which are no longer supported have such low production volumes that their removal from this FGE hardly affects the total annual production volume.

EFFA, 2005b; EFFA, 2006a; EFFA, 2006b; EFFA, 2008; Flavour Industry, 2006). For the 18 supporting substances the total annual volume of production in Europe is approximately 330 000 kg. *d*-Limonene [FL-no: 01.045] accounts for 280 000 kg and 47 000 kg is accounted for by seven other supporting substances: [FL-no: 01.003, 01.004, 01.005, 01.006, 01.007, 01.008 and 01.020] (JECFA, 2005b).

On the basis of the annual volumes of production reported for the 14 candidate substances, the MSDI values for each of these flavourings have been estimated (Table 7).

Nearly 100 % of the total annual volume of production for the candidate substances is accounted for by two substances [FL-no: 01.001 and 01.046]. The estimated daily *per capita* intake from use as flavouring substance is 4000 and 2100 µg, respectively. The daily *per capita* intakes for each of the remaining substances are less than 28 µg (Table 7).

### 4.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For all candidate substances information on food categories and normal and maximum use levels[14,15] were submitted by the Flavour Industry (EFFA, 2005a; EFFA, 2006a; EFFA, 2007, EFFA, 2015). For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

**Table 4:** Use of Candidate Substances

| Food category | Description | Flavourings used |
|---|---|---|
| 01.0 | Dairy products, excluding products of category 2 | 14 |
| 02.0 | Fats and oils, and fat emulsions (type water-in-oil) | 14 |
| 03.0 | Edible ices, including sherbet and sorbet | 14 |
| 04.1 | Processed fruits | 14 |
| 04.2 | Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds | None |
| 05.0 | Confectionery | 14 |
| 06.0 | Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery | 14 |
| 07.0 | Bakery wares | 14 |
| 08.0 | Meat and meat products, including poultry and game | 14 |
| 09.0 | Fish and fish products, including molluscs, crustaceans and echinoderms | 14 |
| 10.0 | Eggs and egg products | FL-no: 01.001, 01.046, 01.070 |
| 11.0 | Sweeteners, including honey | FL-no: 01.001, 01.046, 01.070 |
| 12.0 | Salts, spices, soups, sauces, salads, protein products etc. | 14 |
| 13.0 | Foodstuffs intended for particular nutritional uses | 14 |
| 14.1 | Non-alcoholic ("soft") beverages, excl. dairy products | 14 |
| 14.2 | Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts | 14 |
| 15.0 | Ready-to-eat savouries | 14 |
| 16.0 | Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15 | 14 |

[14] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002).

[15] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

According to the Flavour Industry the normal use levels for the 14 candidate substances, for which use levels have been provided, are in the range of 1 - 20 mg/kg food, and the maximum use levels are in the range of 1 - 100 mg/kg (EFFA, 2002; EFFA, 2005a; EFFA, 2006a; EFFA, 2007, EFFA, 2015) Table B.1.2, Appendix B.

The mTAMDI values are for 11candidate substances from structural class I (see Table 6) above the threshold for structural class I of 1800 µg/person per day. For limonene [FL-no: 01.001] the mTAMDI is 1800 µg/person per day and for each of l-limonene and 1-octene [FL-no: 01.046 and 01.070] the mTAMDI is 1600 µg/person per day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 7 and Appendix B.

## 5. Absorption, Distribution, Metabolism and Elimination

Generally, the available data indicate that the aliphatic and alicyclic hydrocarbons may participate in similar metabolic pathways. Being lipophilic and of relatively low molecular weight, these hydrocarbons may be assumed to be absorbed in the gastrointestinal tract. Subsequently, they can be oxidised to polar oxygenated metabolites, e.g. by CYP-450 enzymes. The phase I metabolites can then be conjugated and excreted mainly in the urine. The candidate and supporting substances are expected to be metabolised either by side chain oxidation or epoxidation of the exocyclic or endocyclic double bonds. Alkyl oxidation initially yields hydroxylated metabolites that may be excreted in conjugated form or undergo further oxidation, yielding more polar metabolites, which can also be excreted. If a double bond is present, intermediate epoxide metabolites may be formed, which are transformed either by hydrolysis to yield diols or by conjugation with glutathione to yield mercapturic acid derivatives. The saturated alkanes in this group may be anticipated to be metabolised via omega and omega-1, -2, -3 or -4 oxidation. Whereas omega oxidation would ultimately lead to the formation of carboxylic acids, the other oxidations would give rise to secondary alcohols and ketones. The carboxylic acids may be expected to participate in the endogenous fatty acid metabolism.

However, for most of the four subgroups (see Appendix C) the information available was scarce and the similarity between candidate and supporting substances was limited. In addition, proper mass balance data were not available. The few mass balance data available indicated only slow elimination. For several subgroups no data were available at all. In Table 5 the final conclusion for each of the candidate substances have been presented together with a brief explanatory statement. For subgroup III there are only data for one supporting substance, *d*-limonene [FL-no: 01.045], which is oxidised in both side chains to yield alcohols and carboxylic acids, which may be conjugated and eliminated in the urine.

A more detailed description of the metabolism is given in Appendix C.

**Table 5:** Can Innocuous Metabolites be Expected to be Formed Based on Available Data?

| FL-no: | Substance name | Innocuous metabolites? |
|---|---|---|
| **Subgroup I: ACYCLIC ALKANES** | | |
| 01.033 | 2,2-Dimethylhexane | Yes |
| 01.034 | 2,4-Dimethylhexane | Yes |
| 01.038 | Dodecane | Yes |
| 01.054 | Pentadecane | Yes |
| 01.057 | Tetradecane | Yes |
| **Subgroup II: ACYCLIC ALKENES** | | |
| 01.035 | 2,6-Dimethylocta-2,4,6-triene | No |
| 01.064 | cis-3,7-Dimethyl-1,3,6-octatriene | No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options) |
| 01.070 | 1-Octene | No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options) |
| **Subgroup III: CYCLOHEXENE HYDROCARBONS** | | |
| 01.027 | Bisabola-1,8,12-triene | Yes |
| 01.028 | β-Bisabolene | Yes |
| 01.039 | delta-Elemene | Yes |
| 01.001 | Limonene | Yes |
| 01.046 | l-Limonene | Yes |
| **Subgroup IV: AROMATIC HYDROCARBONS** | | |
| The substances previously allocated to this subgroup are no longer supported for use as flavouring substances in Europe by Industry | | |
| **Subgroup V: BICYCLIC, NON-AROMATIC HYDROCARBON** | | |
| 01.059 | 4(10)-Thujene | No |
| **Subgroup VI: MACROCYCLIC, NON-AROMATIC HYDROCARBONS** | | |
| The substances previously allocated to this subgroup are no longer supported for use as flavouring substances in Europe by Industry | | |

## 6. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 7.

For the safety evaluation of the 14 candidate substances from chemical group 31, the Procedure as outlined in Appendix A was applied, based on the MSDI approach. The stepwise evaluations of these 14 substances are summarised in Table 7.

Step 1

All 14 candidate substances evaluated using the Procedure are classified into structural class I [FL-no: 01.001, 01.027, 01.028, 01.033, 01.034, 01.035, 01.038, 01.039, 01.046, 01.054, 01.057, 01.059, 01.064 and 01.070], according to the decision tree approach presented by Cramer *et al*. (Cramer et al., 1978).

Step 2

On the basis of the metabolism information available, candidate substances of subgroup I [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] and candidate substances of subgroup III [FL-no: 01.001, 01.027, 01.028, 01.039 and 01.046] may be predicted to be metabolised to innocuous products at the estimated levels of intake based on the MSDI approach. Accordingly the evaluation of these 10 substances, all belonging to structural Cramer class I, will proceed along the A-side of the Procedure scheme.

Two candidate substances from subgroup II [FL-no: 01.064 and 01.070] contain terminal double bonds in the absence of other functional groups that may provide alternative routes of detoxication. Therefore, for these two substances it cannot be concluded that they will be metabolised to innocuous products, and accordingly they proceed along the B-side of the Procedure scheme.

For the remaining two candidate substances [FL-no: 01.035 and 01.059] there are not sufficient data available on biotransformation to conclude that they will be metabolised to innocuous products, and therefore their evaluation will proceed along the B-side of the Procedure scheme.

Step A3

The five candidate substances from subgroup I [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] and three candidate substances from subgroup III [FL-no: 01.027, 01.028 and 01.039], proceeding via the A-side, have been assigned to structural class I and have estimated European daily *per capita* intakes ranging from 0.012 to 2.7 µg (Table 6). These intakes are below the threshold of concern of 1800 µg/person per day for structural class I. Accordingly, it is concluded that these eight candidate substances do not pose a safety concern as flavouring substances when used at estimated levels of intake, based on the MSDI approach. Two candidate substances from subgroup III [FL-no: 01.001 and 01.046] have an estimated European daily *per capita* intakes of 4000 and 2100 µg, respectively, which are above the threshold of concern of 1800 µg/person per day for structural class I. These two candidate substances will therefore proceed to step A4 of the Procedure scheme.

Step A4

The candidate substances [FL-no: 01.001 and 01.046] or their metabolites are not endogenous.

Step A5

The two candidate substances [FL-no: 01.001 and 01.046] are supported by the substance [FL-no: 01.045] for which an adequate carcinogenicity study is available. From this study a no observed adverse effect level (NOAEL) of 215 mg/kg bw per day can be derived. The estimated daily *per capita* intake is 4000 µg for [FL-no: 01.001] and 2100 µg for [FL-no: 01.046], corresponding to 0.07 mg/kg bw per day and 0.035 mg/kg bw per day at a body weight of 60 kg, respectively. Thus, a margin of safety of 3070 can be calculated for [FL-no: 01.001] and a margin of safety of 6140 can be calculated for [FL-no: 01.046]. These two substances are accordingly not expected to be of safety concern at the estimated levels of intake.

Step B3

The four candidate substances [FL-no: 01.035, 0.059, 01.064 and 01.070] proceeding via the B-side and which have been assigned to Cramer structural class I have estimated European daily *per capita* intakes between 0.0085 and 14 µg (Table 6). These intakes are below the threshold of concern of 1800 µg/person per day for structural class I. Accordingly, these four substances proceed to step B4 of the Procedure.

Step B4

For one of these substances, 4(10)-thujene [FL-no: 01.059] a margin of safety could be calculated based upon a NOAEL (222 mg/kg bw per day) for the supporting substance ß-caryophyllene [FL-no: 01.007]. Compared to the MSDI of 4(10)-thujene of 14 µg/*capita* per day equal to 0.2 µg/kg bw per day, the NOAEL provides a margin of safety of $9.5 \times 10^5$.

For the three remaining substances, 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070] margins of safety could be calculated based upon a NOAEL (44 mg/kg bw per day) for the supporting substance myrcene [FL-no: 01.008]. Compared to the MSDI of 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070] of 9.1, 14 and 0.0085 µg/*capita* per day equal to 0.15, 0.23 and 0.00014 µg/kg bw per day, the NOAEL provides margins of safety of $2.9 \times 10^5$, $1.9 \times 10^5$ and $3.1 \times 10^8$.

## 7. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

For 11 of the 14 candidate substances, the mTAMDIs are above the threshold for structural class I of 1800 µg/person per day. For limonene [FL-no: 01.001] the mTAMDI is 1800 µg/person per day and for each of l-limonene and 1-octene [FL-no: 01.046 and 01.070] the mTAMDI is 1600 µg/person per day.For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 6.

For 11 of the 14 candidate substances, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

**Table 6:** Estimated intakes based on the MSDI approach and the mTAMDI approach

| FL-no | EU Register name | MSDI (µg/*capita*/day) | mTAMDI (µg/person/day) | Structural class | Threshold of concern (µg/person/day) |
|---|---|---|---|---|---|
| 01.027 | Bisabola-1,8,12-triene | 0.024 | 3900 | Class I | 1800 |
| 01.028 | beta-Bisabolene | 2.7 | 3900 | Class I | 1800 |
| 01.033 | 2,2-Dimethylhexane | 1.2 | 3900 | Class I | 1800 |
| 01.034 | 2,4-Dimethylhexane | 1.2 | 3900 | Class I | 1800 |
| 01.038 | Dodecane | 0.012 | 3900 | Class I | 1800 |
| 01.039 | delta-Elemene | 0.012 | 3900 | Class I | 1800 |
| 01.054 | Pentadecane | 0.61 | 3900 | Class I | 1800 |
| 01.057 | Tetradecane | 0.012 | 3900 | Class I | 1800 |
| 01.035 | 2,6-Dimethylocta-2,4,6-triene | 9.1 | 3900 | Class I | 1800 |
| 01.059 | 4(10)-Thujene | 14 | 3100 | Class I | 1800 |
| 01.064 | cis-3,7-Dimethyl-1,3,6-octatriene | 14 | 3900 | Class I | 1800 |
| 01.070 | 1-Octene | 0.0085 | 1600 | Class I | 1800 |
| 01.001 | Limonene | 4000 | 1800 | Class I | 1800 |
| 01.046 | l-Limonene | 2100 | 1600 | Class I | 1800 |

## 8. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

The combined intakes have been calculated on the basis of the annual production volumes from use as flavouring substances in Europe (EFFA, 2005a; EFFA, 2005b; EFFA, 2006a; EFFA, 2006b; Flavour Industry, 2006; JECFA, 2005b).

Subgroup I (acyclic alkanes): The combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Appendix A), is 3.0 µg/*capita* per day, which does not exceed the threshold of 1800 µg/person per day. There are no supporting substances in subgroup I.

Subgroup II (acyclic alkenes): The combined intake of the three candidate substances, all from structural class I and evaluated via the B-side of the Procedure (Appendix A), is 23 µg/*capita* per day, which does not exceed the threshold of 1800 µg/person per day. Subgroup III (cyclohexene hydrocarbons): The combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Appendix A), is 6100 µg/*capita* per day, which exceeds the threshold of 1800 µg/person per day. The total combined intake of the five candidate and six supporting substances (also from structural class I) is approximately 42000 µg/*capita* per day. This intake exceeds the threshold of 1800 µg/person per day for a structural class I substance. However, together, limonene [FL-no: 01.001], *l*-limonene [FL-no: 01.046] and *d*-limonene (supporting substance [FL-no: 01.045]) account for approximately 40000 µg/*capita* per day. The total combined intake of 42000 µg/*capita* per day for the candidate and the supporting substances corresponds to 700 µg/kg bw per day for a person with a body weight of 60 kg. Thus, based on the NOAEL for *d*-limonene of 215 mg/kg bw per day, derived from a chronic 2-year toxicity study, a margin of safety of 307 can be calculated, and accordingly these substances are not expected to be of safety concern at the estimated level of intake.

Subgroup V (bicyclic, non-aromatic hydrocarbons): The total combined intake of the one candidate and eight supporting substances, all from structural class I and evaluated via the B-side of the Procedure (Appendix A) is approximately 3800 µg/*capita* per day. This intake exceeds the threshold of 1800 µg/person per day for a structural class I substance. However, together, three supporting substances, pin-2(10)-ene [FL-no: 01.003], pin-2(3)-ene [FL-no: 01.004] and β-caryophyllene [FL-no: 01.007] account for approximately 3400 µg/*capita* per day. The total combined intake of 3800 µg/*capita* per day for the candidate and the supporting substances corresponds to 63 µg/kg bw per day for a person with a body weight of 60 kg. Thus, based on the NOAEL for β-caryophyllene of 222 mg/kg bw per day, a margin of safety of 3500 can be calculated, and accordingly these substances are not expected to be of safety concern at the estimated level of intake.

## 9. Toxicity

### 9.1. Acute Toxicity

Data are available for two candidate substances, 15 supporting substances and one structurally related substance (1-methyl cyclohexa-1,3-diene [former FL-no: 01.077]). Oral $LD_{50}$ values in rat and mouse ranged from 1590 to 13 000 mg/kg body weight (bw).

The acute toxicity data are summarised in Table 9.

### 9.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data are available for two supporting substances from subgroup II (myrcene [FL no: 01.008] and undeca-1,3,5-triene [FL no: 01.061], for one supporting substance from subgroup III (*d*-limonene [FL no: 01.045]), for three supporting substances for subgroup V (pinene [FL no: 01.003], β-caryophyllene [FL-no: 01.007] and camphene [FL no: 01.009]) (see also Table 10). In the text below the relevant studies are discussed.

*Subgroup II*

*Myrcene [01.008]*

*Mice, 90 day study (NTP, 2010)*

Male and female $B6C3F_1$ mice (10/sex/group) were administered 0, 250, 500, 1000, 2000 or 4000 mg/kg bw of myrcene by gavage 5 days a week for 14 weeks. Body weights and clinical observations were recorded weekly. Blood was collected from mice surviving to the end of the study for haematological analyses and micronuclei evaluation. Sperm morphology and vaginal cytology evaluations were conducted at the end of the study on animals in the control and three lowest dose groups. At necropsy, organ weights were measured and complete histopathological examination was performed on animals from the dose groups 0, 1000, 2000 and 4000 mg/kg bw, and all animals that died early.

All animals in the 4000 mg/kg bw group died within the first three days while 9 of 10 males and 8 of 10 females in the 2000 mg/kg bw group died prior to week 5. In animals that died prior to study termination, clinical signs included lethargy, abnormal breathing and/or thin appearance. The final mean body weights and body weight gain of the 1000 mg/kg bw males and 500 mg/kg bw females were significantly less than those of vehicle controls. Because of the low survival in the two top doses, further results are not reported for those doses.

A significant, approximately 15-20 % decrease in hematocrit, haemoglobin and erythrocyte count values was observed in the 1000 mg/kg bw dose group in both females and males at week 14. A dose-dependent significant increase in the relative liver weight (approximately 7 %, 6 % and 17 %) were observed for all doses in male mice, and a significant increase in absolute liver weight were observed for the low dose males only. In female mice a dose-dependent significant increase in relative kidney weight was observed at all doses (approximately 14 %, 12 % and 22 %), with a significant increase in absolute kidney weight in the 1000 mg/kg bw dose group only. Also for female mice dose-dependent increases in absolute and relative liver weight (approximately 8 %, 17 % and 26 %) were observed, but the increase reached statistical significance only at 500 and 1000 mg/kg bw. There were no significant changes seen in the weights of the reproductive organs, in the sperm parameters, or in oestrous cyclicity at any dose level. No significant histopathological changes in other organs examined, including kidney, were observed in mice receiving up to 1000 mg/kg bw myrcene for 14 weeks.

As a significant dose-dependent increase in the relative kidney weight was observed for female mice at all treatment doses, no NOAEL for this study could be allocated.

*Rats, 90-day study (NTP, 2010)*

Male and female F344N Fisher rats (10/sex/group) were administered 0, 250, 500, 1000, 2000 or 4000 mg/kg bw of myrcene by gavage 5 days a week for 14 weeks. Body weights and clinical observations were recorded weekly. Blood was collected from rats surviving to the end of the study for clinical chemistry and haematological analyses. Sperm morphology and vaginal cytology evaluations were conducted at the end of the study on animals in the control and three lowest dose groups. At necropsy, organ weights were measured and complete histopathological examination was performed on animals from the dose groups 0, 2000 and 4000 mg/kg bw, and all animals that died early. Tissues were examined in the lower dose groups to a no-effect level, including renal pathology in all dose groups. Additionally sections of kidney from both sexes were stained using the Mallory-Heidenhain technique for investigation of hyaline droplet formation (indicative of development of α2μ-globulin).

All animals in the group receiving 4000 mg/kg bw myrcene died within the first week of the study, except for one male that died at day 11. One male receiving 500 mg/kg bw, one male and one female receiving 1000 mg/kg bw and two males and four females receiving 2000 mg/kg bw died before the end of the study. Final mean body weight and mean body weight gains of males and females administered 500 mg/kg bw or more were significantly less than those of vehicle control. At termination at week 14, dose-related decreases in plasma creatinine concentration in both males and females were observed, statistically significant at 500 mg/kg bw and above in males and at 250 mg/kg bw and above in females. These decreases were suggested by the authors to be associated with the decreased body weight gains observed in treated rats. No other consistent changes in clinical chemistry parameters were found.

Absolute kidney and liver weights were significantly increased in both male and female rats receiving myrcene. Also a dose-dependent increase in the relative liver and kidney weights were observed for males (25-150 % in kidney, 13-46 % in liver) and females (27-100 % in kidney, 13-67 % in liver). Microscopically, the incidence of renal tubular necrosis was significantly increased in all dosed groups of males and females, with increasing severity from minimal to moderate related to dose. Both control and treated rats showed development of chronic progressive nephropathy (CPN), although the incidence was higher in treated rats. Treatment-related increases in the incidences and severity of hyaline droplet accumulation were found in the 250, 500 and 1000 mg/kg bw males, accompanied by granular casts in the outer medulla of the kidney. Hyaline droplet formation was not observed in the 2000 mg/kg bw males, although the animals showed a high incidence of renal tubular necrosis, nephrosis and CPN. No evidence of hyaline droplet accumulation was found in female rats, however treated females showed both nephrosis and CPN. A significant increase in nephrosis was observed in the 1000 and 2000 mg/kg bw dose groups of both males and females, with a dose-related increase in severity from minimal to moderate. Nephrosis is an uncommon lesion defined as renal tubule epithelial degeneration and regeneration.

A dose-related effect of myrcene in the nose was observed in both sexes as degeneration of the olfactory epithelium and necrosis of the respiratory epithelium (significant only at 2000 mg/kg bw) accompanied by chronic inflammatory change (significant at 1000 and 2000 mg/kg bw).

The incidence of splenic atrophy were significantly increased in both sexes receiving 2000 mg/kg bw, accompanied by thymic necrosis in one male and three females. In the mesenteric lymph node, the incidence of atrophy were increased in males receiving 2000 mg/kg bw and females receiving 1000 or 2000 mg/kg bw. The lymphoid changes in these organs were considered by the authors to be secondary to morbidity rather than a direct toxic effect of myrcene.

It has been argued that increased hyaline droplet accumulation in male rats is characteristic of α2μ-globulin nephropathy (Hard et al., 1993), which is a male rat specific effect with little relevance for humans. The evidence provided for the mechanistic background of the hyaline droplet formation was considered too limited to completely disregard the nephrotoxic effects in male rats as irrelevant for humans. Involvement of α2μ-globulin accumulation (e.g. by immunohistochemical techniques) was not demonstrated. In addition, also renal toxicity was observed in the female animals. Therefore, based on the presence of renal tubular necrosis in all test groups, a NOAEL could not be assigned.

*Mice, 2-year carcinogenicity study (NTP, 2010)*

Groups of $B6C3F_1$ mice (50/sex/group) were administered 0, 250, 500 or 1000 mg myrcene/kg bw per day in corn oil by gavage once per day, five days a week for 104 (females) weeks or 105 weeks (males). The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Complete necropsies and microscopic examination were performed on all animals. Histological examinations were performed on all animals and tissues from all major organs were examined.

Mean body weights of males receiving 1000 mg/kg bw and females receiving 500 or 1000 mg/kg bw were less than those of the vehicle control. Survival of the high-dose group was significantly reduced for both males and females. Due to the high mortality in the 1000 mg/kg bw dose group, the results from this group are not described further.

In the liver there was an increase in hepatocellular carcinoma in both males and females, with a significant increase in incidence in males at the 500 mg/kg bw dose level (incidence 1 in controls, 4 in the 250 mg/kg bw group and 9 in the 500 mg/kg bw group) and females at 250 mg/kg bw, but not at 500 mg/kg bw. Males and to a lesser extent females showed an increase in hepatocellular adenoma in the liver at both 250 and 500 mg/kg bw. Liver hypertrophy was observed to increase with dose both in incidence and severity, reaching statistical significance only at 500 mg/kg bw in both males and females. Eosinophilic foci and cytoplasmic vacuolization were noted in both male and female treatment groups.

Treatment-related changes in other organs included increases in hyperplasia, inflammation, necrosis and ulcer of the forestomach, which were most likely attributable to gavage administration of an irritant substance. Bone marrow atrophy and mandibular and mesenteric lymph node atrophy was observed in both males and females at the 500 mg/kg bw dose. In addition, male mice showed atrophy of the spleen at the 500 mg/kg bw dose.

Taking into account the high sensitivity of this mice strain to tumour development in the liver, these effects were not regarded relevant to humans. A NOAEL of 250 mg/kg bw for myrcene was allocated, based on the increase in bone marrow atrophy and lymph node atrophy observed in both males and females at 500 mg/kg bw dose.

*Rats, two-year carcinogenicity study (NTP, 2010)*

A chronic two-year bioassay on myrcene using the standard NTP protocol with F344/N rats was conducted. Doses were determined from the results of the prior 13-week subchronic study. Groups of F344/N rats (50/sex/group) were administered 0, 250, 500 or 1000 mg myrcene/kg bw per day in corn oil by gavage once per day, five days a week for 104 weeks. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Complete necropsies and microscopic examination were performed on all animals. Histological examinations were performed on all animals and tissues from all major organs were examined.

Survival rates of females were comparable across all control and treatment groups. Survival of males in the low- and mid-dose groups was similar to that in the controls. However, no males in the high-dose group survived past 83 weeks of the study, due to renal toxicity. Body weight gain was significantly reduced in high-dose males and females, while the mean body weight of the 500 mg/kg females were less than those of the vehicle controls during much of the study but were similar by the end of the study. Due to the high mortality of males in the 1000 mg/kg bw group, the data from this dose group are not presented further.

The incidences of renal tubular adenoma and of renal tubule adenoma or carcinoma (combined) showed a significant and dose-dependent increase in the 250 and 500 mg/kg bw males. Two renal tubule adenomas occurred in the 1000 mg/kg bw females, the incidence being higher than the

historical control mean for the laboratory. In males high increase in both the incidence and severity of renal tubular nephrosis were observed, already at the lowest dose, with 84 and 92 % incidence at 250 and 500 mg/kg bw, respectively. A significant but not dose related increase in papilla mineralisation, epithelial hyperplasia and inflammation was seen in the dose groups 250 and 500 mg/kg bw. In female rats a dose-dependent increase in nephropathy (CPN) were observed for the doses 250, 500 and 1000 mg/kg bw and a dose-dependent increase in the incidence and severity of renal tubule nephrosis at the doses 500 and 1000 mg/kg bw. In addition a non-dose related significant increase in renal tubular hyperplasia was reported at the doses (250, 500, 1000 mg/kg bw). Nephrosis, observed both in male and female rats, was reported by the authors to be an uncommon lesion defined as renal tubule epithelial degeneration and regeneration. This indicates that myrcene might cause nephrotoxicity by a mechanism other than, or in addition to, α2μ-globulin nephropathy. The observation of renal neoplasms in female rats also suggests a mechanism of carcinogenesis that may be related to nephrosis and is distinct from the α2μ-globulin mechanism.

Other histopathological changes seen in the study included chronic active inflammation in the nose and forestomach, suggesting that the substance is an irritant. In females, the incidence of thyroid gland C-cell adenomas was significantly increased in the 250 mg/kg bw group, but the incidence did not increase with increasing dose. In the lung, the incidence of alveolar/bronchiolar adenoma in the 250 mg/kg bw group exceeded the control means, but was not observed at higher doses.

Due to the observation of renal tubular adenomas and carcinomas in all dose groups in male rats, accompanied by an increased incidence and severity of nephrosis in both sexes, a NOAEL for myrcene from this study could not be allocated.

*Overall conclusion for the NTP study on myrcene*

No overall NOAEL from the NTP study on myrcene could be allocated due to the observation renal toxicity in male and female rats at all dose groups. The Panel has considered deriving a BMDL from the NTP study of myrcene. However, a BMDL from this study could not be derived because of a lack of dose-response since nearly 100 % incidence of nephropathy was observed in rats already at the lowest dose of myrcene.

Since the publication of FGE.25Rev2 (EFSA CEF Panel, 2011) new data on myrcene have become available (Bauter, 2013b). The results are described in more detail in FGE.78Rev 2 (EFSA CEF Panel, in press).

In an OECD 408 compliant 90-day study the subchronic toxicity of myrcene (93.3 % pure) was evaluated in male and female rats, based on daily exposure to the test substance in the diet (Bauter, 2013b). Four groups of adult Sprague-Dawley rats (10/sex/group) were maintained on diets prepared to contain 0, 700, 2100 or 4200 mg/kg feed of myrcene. However, the myrcene content of the diet decreased considerably over 7 days, so that every week a new batch of the diet was prepared. Therefore, the Panel decided to take the concentration of myrcene in the diet on the last day of the week for quantification of the exposure, rather than the (logarithmic) mean over the whole week. Accordingly, an adjusted calculated mean daily intake of 8.0, 40 and 44 mg/kg bw per day, respectively for males, and 9.6, 48 and 53 mg/kg bw per day, respectively, for females for 90 days was calculated.

There were no mortalities, clinical, or ophthalmological changes attributable to myrcene administration. There were no statistically significant, dietary concentration-dependent changes in body weight, body weight gain, food consumption, or food efficiency in males and females attributed to the administration of myrcene during the study.

There were no clinical pathological findings, changes in macroscopic or microscopic histopathology, or organ weight changes in the groups administered myrcene. Some incidental changes in clinical chemistry and hematology parameters were within approximate historical control values, did not

correlate with macroscopic or histopathological findings, were without biologic impact, and were considered not toxicologically relevant. A few histopathological changes were considered incidental, spontaneous in nature as observed for the age and strain of rat used in this study, and had no established relationship to administration of the test substance.

Under the conditions of the study and based on the toxicological endpoints evaluated, the no-adverse-effect level (NOAEL) for administration of myrcene in the diet was determined to be the highest dose-group, calculated to provide an estimated daily intake of 44 mg/kg bw per day for males and 53 mg/kg bw per day for females, respectively.

The Panel took note of the fact that two 90-day studies are available on myrcene: the NTP study and the study by Bauter (2013b). While the NTP study showed kidney toxicity in all animals at 250 mg/kg body wt per day, the study by Bauter (2013b) observed no toxicity at all in the kidneys at 44 and 53 mg/kg bw per day in males and females resp. The Panel decided to accept the NOAEL of the study by Bauter (2013b) (44 mg/kg bw per day) because the NTP study is a gavage study, which leads to peak exposure levels compared to the Bauter study in which the compound was added in the food. Thus, the Bauter study reflects the dietary administration (consumption with food matrix, over a more extended period of time) in consumers much better than gavage (NTP) study. Additionally, in the Bauter study no kidney toxicity was observed at all and the NOAEL derived from the Bauter study is six-fold lower than the effect level in the NTP study.

*Subgroup III*

*d-Limonene [FL-no: 01.045]*

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated the supporting substance *d*-limonene as an additive in its forty-first meeting in 1993, and withdrew the previous ADI for *d*-limonene and allocated an ADI "not specified" (JECFA, 1993). This assessment was mainly based on an NTP study with *d*-limonene from 1990 (NTP, 1990). In the carcinogenicity study, F344 rats (n = 50/dose/sex) were treated with 0, 75 and 150 mg/kg *d*-limonene (males) and 0, 300 and 600 mg/kg (females) by gavage in corn oil for five days a week. B6C3F$_1$ mice (n = 50/dose/sex) were treated with 0, 250 and 500 mg/kg *d*-limonene (males) and 0, 500 and 1000 mg/kg (females) in corn oil by gavage for five days a week. In the high dose female rats, the survival was reduced. No effect on survival or any other toxic effect was observed in the females of the low dose group. The major toxicological effect in the male rats was found in the kidneys where a dose dependent increase in both renal mineralization and epithelial hyperplasia, and a dose-dependent increase in renal tubular cell adenomas and adenocarcinomas was observed. These effects were accompanied with dose related increase in α2μ-globulin in the kidney of male rats. No increase in α2μ-globulin, kidney nephropathy or renal adenomas or carcinomas was found in female rats. Therefore, the toxicological effects seen in the male rat kidneys are due to α2μ-globulin accumulation related nephropathy specifically seen in male rats, which is not relevant for humans (Hard et al., 1993). A reduction in the mean body weights at the high dose group was observed for male and female rats (4 – 7 %) and high dose female mice (5-15 %). For the male rats this reduction in mean body weight may have been linked to the toxicity in the kidneys. Based on the decreased body weights in female mice, a NOAEL of 500 mg/kg bw per day (5 days/week) could be derived, but considering the decrease in survival in the female rats exposed at 600 mg/kg bw per day (5 days/week) an overall NOAEL of 300 mg/kg bw per day (5 days/week) should be derived from these NTP studies. This would correspond to 215 mg/kg bw per day for daily exposure.

*Subgroup V*

*Supporting substance*

*ß-Caryophyllene [FL-no: 01.007]*

In a 14 day range finding dietary study (Bauter, 2011), groups (3/sex/dietary intake level) of male and female Hsd:SD® rats were fed a diet designed to provide 0 (dietary control), 6000, 18 000 and 48 000 mg/kg feed of β-caryophyllene daily. These estimated dietary levels correspond to the measured intakes of 0, 516, 1547 and 3569 mg/kg bw per day for males and 0, 528, 1582 and 4438 mg/kg bw per day for females, respectively. Clinical observations were recorded daily and body weights and food consumption observations were made on days 0, 7 and 14. There were no mortalities. Hyperactivity observed in 1/3 (33 %) of males and females in group 4 (48 000 mg/kg feed) in the latter part of the study may be possibly attributed to test substance administration. Dose-dependent decreases in male food consumption and food efficiency with significant corresponding decreases in group 4 male body weight and body weight gain were considered a result of test substance administration, but were not correlated with any other clinical signs. Females did not exhibit significant differences from female control. Findings at terminal sacrifice included all (100 %) males and females of group 4 with distention (cecum) and slight redness of the stomach, small intestines, and cecum. Based upon the limited toxicological endpoints evaluated, the study authors selected doses for the 90-day study.

90-Day Study on β-Caryophyllene [FL-no: 01.007]

In an OECD 408 compliant 90-day study, 4 groups of rats (10/sex/dietary intake level) of male and female CRL Sprague-Dawley CD®IGS rats were fed a diet designed to provide 0 (dietary control), 3500, 7000 and 21 000 mg/kg feed and 3500, 14 000 and 56 000 mg/kg feed of β-caryophyllene for males and females, respectively, daily (Bauter, 2013a). These dietary levels corresponded to measured daily intakes of 0, 222, 456 and 1367 mg/kg bw for males and 0, 263, 1033 and 4278 mg/kg bw for females, respectively (Bauter, 2013a). The purity of the β-caryophyllene preparation was between 99 % (start of study) and 96 % (end of study). Clinical observations of toxicity were performed on day 0 and weekly until sacrifice. Animals were weighed on day 0 at the start of the study and weekly thereafter. Food consumption and efficiency were measured and calculated weekly. Blood chemistry and haematology were performed on blood drawn via sublingual bleed during week 12 after overnight fast. Urine was collected during the 15 hours prior to the blood draw. Prior to initiation of the study and on day 91 the eyes of all rats were examined by focal illumination and indirect ophthalmoscopy. At termination of the study all survivors were sacrificed and subject to full necropsy.

There were no mortalities, clinical signs of toxicity or ophthalmological changes associated with the presence of β-caryophyllene in the diet. There were statistically significant and concentration-related reductions in body weight gain, food consumption and food efficiency in males and females at the 21 000 mg/kg feed and 56 000 mg/kg feed concentration groups, respectively (body weight of high dose groups: males 77.3 %, females 82.6 % as compared to controls).

Although for some parameters statistically significant differences were found when compared to concurrent controls, haematology, clinical chemistry, coagulation and urine analysis parameters for the middle and high concentrations for both males in general were within the range of historical controls. Most of these changes were small, and observed in the highest dose group. Thus, in the female test groups, a statistically significant increase for platelet count was reported at the highest dietary level; such an increase was not observed in the male test groups. A dose dependent increase in white blood cells in males reached statistical significance at the middle and high dose; several other blood cells showed significant changes at the highest dose as well in males; the effects in females were less pronounced. There were no histopathology findings correlating to these variations.

In females a dose-dependent decrease in serum glucose concentrations and an increase in triglyceride levels reached statistically significance only at the highest dose level. In conjunction with changes reported in the liver these changes were attributed to metabolic changes as a result of high concentrations of β-caryophyllene in the diet. Pathological findings include increases in absolute and relative liver weights; these were found statistically significant in the mid- and high-dose groups of both sexes. Histopathological liver changes at the mid and high intake levels for both sexes were characterized by centrilobular to midzonal distributed hepatocellular hypertrophy. Based on hepatocyte hypertrophy in both sexes, the increases in absolute and/or relative liver weights at the mid- and highest dietary levels, and the absence of any other significant abnormality upon histopathological examination, the study authors conclude that the hepatocyte hypertrophy is the result of hepatic enzyme induction; this has, however, not been confirmed by measurements of relevant enzymes.

Necropsy revealed enlarged kidneys in one male at the highest test concentration and significant increases in relative kidney weight of male high-dose as well as of female mid- and high-dose groups. Microscopic examination revealed mononuclear infiltration in only one of ten females of the highest dose group. No other microscopically visible alterations were reported in the kidneys of female rats. The increase in kidney weights in female remains unexplained. Microscopical examination of the kidney of males revealed an increased incidence of nephropathy characterised by regeneration of proximal cortical tubules with thickened membranes, mononuclear cell infiltration and tubular casts at all dose levels. The severity of the nephropathy exhibited a dose-dependent shift from low to high grades. Kidney cells of affected males also were reported to have necrotic nuclei and an increase in eosinophilic cytoplasm. Likewise, in the kidneys of all treated males eosinophilic cytoplasmic droplets were present, with a dose-dependent shift to higher grades In a supplementary study the kidney slides of exclusively male rats were stained also with Mallory Heidenhain stain (Zook and Garlick, 2013), which results in enhanced staining of the cytoplasmic droplets (De Rijk et al., 2003; Frazier et al., 2012); this confirmed the observations of the eosine staining. An increased hyaline droplet accumulation in male rats exclusively is characteristic of $\alpha_{2u}$-globulin nephropathy (Hard et al., 1993), which is considered a male rat specific effect with little relevance for humans. Although no specific immunohistochemical staining of $\alpha_{2u}$ –globulin has been done to confirm the presence of this protein, the Panel considers the evidence sufficient to conclude that this kidney toxicity in male rats exclusively is not relevant for humans.

Microscopic examination of the mesenteric lymph nodes revealed the presence of erythrocytes in the sinuses at the mid- and high-intake levels for both sexes. Additionally, reduced spleen weights for males at the highest dietary level were considered related to general reductions in lymphoid system weights.

The Panel concluded that under the conditions of the present 90-day dietary toxicity study and based on the toxicological findings in haematology in males, the liver, the mesenteric lymph node pathology in both sexes and non-explained effects in female kidneys only the lowest dose provides a no-adverse-effect level (NOAEL) for β-caryophyllene, which is the lowest in male rats: 222 mg/kg bw per day.

Repeated dose toxicity data are summarised in Table 10.

### 9.3. Developmental / Reproductive Toxicity Studies

No developmental or reproductive studies are available for the candidate substances but for three supporting substances.

The developmental/reproductive toxicity data are summarised in Table 11.

### 9.4. Genotoxicity Studies

Data from *in vitro* tests are available for two candidate substances (subgroup I: [FL-no: 01.038 and FL-no: 01.057]) and 10 supporting flavouring substances (one from subgroup II, four from subgroup

III, and five from subgroup V (for pin-2(3)-ene [FL-no: 01.004] also data for separate stereoisomers were available (+ and -)- α-pinene ), and one structurally related substance, (2-methylbuta-1,3-diene) not used as flavouring substance from subgroup II. Data for three supporting substances [FL-no: 01.008 (subgroup II), FL-no: 01.019 (subgroup III), FL-no: 01.004 (subgroup V), and data for the structurally related substance from subgroup II are considered valid.

Data from *in vivo* tests are available for two supporting substances (one from subgroup II and one from subgroup III) and for one substance structurally related to subgroup II (2-methylbuta-1,3-diene).

*Candidate substances*

*Subgroup I*

The two candidate substances [FL-no: 01.038 and 01.057] tested *in vitro* for bacterial gene mutations gave negative results in bacterial reverse gene mutation tests and for mammalian cell gene mutations.

*Subgroup II*

For the three candidate substances in subgroup II [FL-no: 01.035, 01.064 and 01.070] there are no genotoxicity data available, but it was noted that in contrast to the structurally related substance 2-methyl-1,3-butadiene, these substances do not contain conjugated terminal double bonds, except [FL-no: 01.064].

The available *in vivo* studies on the structurally related substance 2-methylbuta-1,3-diene (isoprene) reported a negative result in a valid chromosomal aberration assay in the bone marrow of mice after 12 days of inhalatory exposure to isoprene. However, isoprene induced sister chromatid exchanges (SCE) in the bone marrow and micronuclei in peripheral blood cells of mice after 12 days of inhalatory exposure in two valid studies carried out within NTP. Induction of micronuclei in peripheral blood cells of mice has also been reported after inhalatory exposure for 13 weeks. In contrast, inhalatory exposure of isoprene to male and female rats for four weeks did not result in an increase in the frequency of micronuclei in the lung fibroblasts. The validity of the latter two studies cannot be evaluated due to limited details available. Isoprene has been reported to bind covalently to haemoglobin *in vivo* (IARC, 1999).

The genotoxic and carcinogenic potential of isoprene has been evaluated by IARC (1999a). It was concluded that there is sufficient evidence of carcinogenicity in experimental mammalians and that isoprene is *'possibly carcinogenic to humans' (Group 2B)* (IARC, 1999). Isoprene has been classified in the EU as a *'Muta. Cat. 3; R68'* and *'Carc. Cat. 2; R45'* (EFSA, 2004b).

The available data on *in vivo* genotoxicity of isoprene indicate a genotoxic potential of the substance. In the light of the evidence of carcinogenic activity of isoprene in rats and mice (NTP, 1999) and the genotoxic effects of isoprene in mice and the fact that the structurally related substance 1,3-butadiene is classified as a genotoxic carcinogen, the Panel concluded that there is reason for concern with respect to genotoxicity and carcinogenicity of isoprene. This substance has been deleted from the Register.

For the supporting substances myrcene, several *in vitro* genotoxicity tests and three *in vivo* genotoxicity studies were available. All the *in vitro* genotoxicity tests on myrcene were negative. Two micronucleus tests on peripheral blood cells and one chromosomal aberration assay with myrcene gave negative results.

*Conclusion on Genotoxicity for subgroup II*

The supporting substance myrcene [FL-no: 01.008] (that is considered by the Panel a more adequate supporting substance for the substances in subgroup II) has like isoprene, two conjugated terminal

double bonds but has a longer chain length, with 10 carbon atoms, like [FL-no: 01.064]. The genotoxicity data available on myrcene do not give rise to concern with respect to genotoxicity. Therefore, the Panel has not concern for genotoxicity for the three substances in subgroup II.

*Subgroup III*

For the five candidate substances in subgroup III no genotoxicity studies were available. For the four supporting substances, *d*-limonene [FL-no: 01.045], gamma-terpinene [FL-no: 01.020], α-terpinene [FL-no: 01.019] and α-phellandrene [FL-no: 01.006], several *in vitro* studies on genotoxicity were available and they were all negative. Also two *in vivo* Comet assay with *d*-limonene and a study with *d*-limonene in BigBlue$^{TM}$ rats were found negative. Therefore, the Panel has no concern for genotoxicity for the substances in subgroup III.

*Subgroup V*

For the candidate substance in subgroup V there are no genotoxicity data available. For the supporting substances, only negative results were reported in the available studies except for delta-3-carene (see Table 12). Delta-3-carene was studied individually as a component in wood fumes and wood fume condensates. A bacterial reverse gene mutation study (insufficiently reported) showed that delta-3-carene induced gene mutations in TA100 and TA102 strains in the absence of metabolic activation at high concentrations only, while it was negative in the presence of metabolic activation (Kurttio et al., 1990).

Information on the supporting substance β-caryophyllene [FL-no: 01.007] has been provided by EFFA (EFFA, 2012). The new data submitted cover a bacterial reverse mutation assay and an *in vivo* mouse erythrocyte micronucleus test.

*In vitro*

No evidence of genotoxic potential was observed when *S. typhimurium* strains TA98 and TA100 and *E. coli* WP2uvrA were incubated with five test concentrations between 2300 and 9000 µg/plate in the presence or absence of rat liver (S9) bioactivation system using the plate incorporation method (Di Sotto et al., 2008). The positive and negative controls provided the appropriate response in the tester strains. However, the study design and reporting exhibits major deviations from OECD guideline 471 and is considered of insufficient quality.

*In vivo*

In an *in vivo* micronucleus induction assay, groups of mice (National Institute of Hygiene, Mexico) (5/sex/dose) were administered a single dose of 0, 20, 200 and 2000 mg/kg bw of β-caryophyllene by corn oil gavage. Blood was drawn and smears for analysis were prepared at 24, 48, 72 and 96 hours post dose. No significant increase in the induction of micronucleated polychromatic erythrocytes (MNPE) was observed for the treatments groups while all positive controls provided the appropriate response (Molina-Jasso et al., 2009). In a follow up study, groups of the same strain of mice (5/sex/dose) were administered daily doses of 0, 20, 200 and 2000 mg/kg bw for three consecutive days by corn oil gavage with blood sampled and smears for analysis prepared at 24, 48, 72 and 96 hours post administration. There was no significant increase in MNPE however there was a slight increase in MNPE at the highest dose tested from 48 - 96 hours post dose. The authors did not consider this an indication of genotoxic potential due to the high dose administered over three consecutive days (Molina-Jasso et al., 2009). The Panel noted that the limit dose (2000 mg/kg) was applied in both treatment regimens without signs of toxicity (altered PCE/NCE ratio). The study is compliant with OCED guideline 474, except the reporting of individual data and historical controls; therefore, this study is considered of limited validity.

Altogether, the Panel has no concern for genotoxicity for the substances in subgroup V.

Data on the genotoxicity of the flavouring substances in this group are limited and the genotoxicity could not be assessed adequately for these substances. However, the Panel concluded that the available data do not preclude evaluating the 14 candidate substances using the Procedure.

Data on genotoxicity are summarised in Table 12 and 13.

**New Mutagenicity/Genotoxicity Studies on β-Caryophyllene [FL-no: 01.007]**

Information on the representative substance β-caryophyllene [FL-no: 01.007] has been provided by EFFA (EFFA, 2012). The new data submitted cover a bacterial reverse mutation assay and an *in vivo* mouse erythrocyte micronucleus test.

*In vitro*

No evidence of genotoxic potential was observed when *S. typhimurium* strains TA98 and TA100 and *E. coli* WP2uvrA were incubated with 5 test concentrations between 2300 and 9000 µg/plate in the presence or absence of rat liver (S9) bioactivation system using the plate incorporation method (Di Sotto et al., 2008). The positive and negative controls provided the appropriate response in the tester strains. However, the study design and reporting exhibits major deviations from OECD guideline 471 (see Table12).

*In vivo*

In an *in vivo* micronucleus induction assay, groups of mice (5/sex/dose; mouse strain from National Institute of Hygiene (NIH), Mexico) were administered by gavage as a single dose of 0, 20, 200 and 2000 mg/kg bw of β-caryophyllene solved in corn oil. Blood was drawn and smears for analysis were prepared at 24, 48, 72 and 96 hours post dose. No significant increase in the induction of micronucleated polychromatic erythrocytes (MNPE) was observed for the treatment groups while all positive controls provided the appropriate response (Molina-Jasso et al., 2009). In a follow up study groups of NIH mice (5/sex/dose) were administered daily doses of 0, 20, 200 and 2000 mg/kg bw for three consecutive days by gavage with blood sampled and smears for analysis prepared at 24, 48, 72 and 96 hours post administration. The Panel noted that the limit dose (2000 mg/kg) was applied in both treatment regimens without signs of toxicity (altered reticulocyte/NCE ratio). A slight, non-significant increase in the MNPE frequency was observed in the highest dose group at 48, 72 and 96 hours, respectively. However, at none of these time points the effects were clearly dose-related since the low and medium doses resulted in lower MNPE frequencies than that observed in control animals. Overall, the Panel concluded that β-caryophyllene did not cause a significant increase in MNPE. However, the study exhibits deviations from OECD guideline 474 and therefore, is considered to be of limited validity (see Table 13).

Although the newly submitted data are of limited validity they do not preclude the substances to be evaluated using the Procedure.

For a summary of *in vitro / in vivo* genotoxicity data on β-caryophyllene, see Table 14.

## CONCLUSIONS

The present revision of FGE.25, Revision 3 includes the assessment of a 90-day study on ß-caryophyllene [FL-no: 01.007] supporting the candidate substance 4(10)-thujene [FL-no: 01.059] and a 90-day study on myrcene [FL-no: 01.008] supporting the candidate substances 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070].

Since the publication of FGE.25Rev2, the Industry has informed EFSA that 23 substances [former FL-no: 01.021, 01.022, 01.023, 01.030, 01.031, 01.032, 01.036, 01.037, 01.042, 01.043, 01.044, 01.047, 01.050, 01.051, 01.052, 01.053, 01.055, 01.056, 01.058, 01.060, 01.066, 01.067 and 01.078] are no longer supported for use as flavouring substances in Europe by Industry. The FGE.25Rev3 therefore deals with 14 flavouring substances in total.

The 14 candidate substances are aliphatic hydrocarbons from chemical group 31, which belong to the subgroups I) acyclic alkanes, II) acyclic alkenes, III) cyclohexene hydrocarbons and V) bicyclic, non-aromatic hydrocarbons.

Seven of the 14 flavouring substances possess chiral centres and three can exist as geometrical isomers.

All of the 14 candidate substances are classified into structural class I, according to the decision tree approach presented by Cramer et al. (1978).

Twelve of the 14 candidate substances have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, 12 of the 14 flavouring substances in this group have intakes in Europe from 0.0085 to 14 µg/*capita* per day, which are below the threshold of concern value for structural class I (1800 µg/person per day) substances. For limonene [FL-no: 01.001] and l-limonene [FL-no: 01.046] the intakes are 4000 and 2100 µg/*capita* per day, which are above the threshold of concern value for structural class I (1800 µg/person per day).

On the basis of the reported annual production volumes in Europe (MSDI approach), the total combined intakes of the candidate and supporting substances can be calculated for the substances in subgroup I , II, III and V.

Subgroup I (acyclic alkanes): the combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Appendix A), is 3.0 µg/*capita* per day, which does not exceed the threshold of 1800 µg/person per day. There are no supporting substances in subgroup I.

Subgroup II (acyclic alkenes): the combined intake of the three candidate substances, all from structural class I and evaluated via the B-side of the Procedure (Appendix A), is 23 µg/*capita* per day, which does not exceed the threshold of 1800 µg/person per day.

Subgroup III (cyclohexene hydrocarbons): the combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Appendix A), is 6100 µg/*capita* per day, which does exceed the threshold of 1800 µg/person per day. The total combined intake of the five candidate and six supporting substances (also from structural class I) is approximately 42 000 µg/*capita* per day. This intake exceeds the threshold of 1800 µg/person per day for a structural class I substance. However, together, limonene [FL-no: 01.001], *l*-limonene [FL-no: 01.046] and *d*-limonene (supporting substance [FL-no: 01.045]) account for approximately 40 000 µg/*capita* per day. The total combined intake of 42 000 µg/*capita* per day for the candidate and the supporting substances corresponds to 700 µg/kg bw per day for a person with a body weight of 60 kg. Thus, based on the NOAEL for *d*-limonene of 215 mg/kg bw per day, derived from a chronic 2-year toxicity study, a

margin of safety of 307 can be calculated, and accordingly these substances are not expected to be of safety concern at the estimated level of intake.

Subgroup V (bicyclic, non-aromatic hydrocarbon): the total combined intake of the one candidate and eight supporting substances, all from structural class I and evaluated via the B-side of the Procedure (Appendix A) is approximately 3800 µg/*capita* per day. This intake exceeds the threshold of 1800 µg/person per day for a structural class I substance. However, together, three supporting substances, pin-2(10)-ene [FL-no: 01.003], pin-2(3)-ene [FL-no: 01.004] and β-caryophyllene [FL-no: 01.007] account for approximately 3400 µg/*capita* per day. The total combined intake of 3800 µg/*capita* per day for the candidate and the supporting substances corresponds to 63 µg/kg bw per day for a person with a body weight of 60 kg. Thus, based on the NOAEL for β-caryophyllene of 222 mg/kg bw per day, a margin of safety of 3500 can be calculated, and accordingly these substances are not expected to be of safety concern at the estimated level of intake.

The available information on metabolism of the 14 candidate substances evaluated through the Procedure or the supporting substances for this FGE was very limited. Overall, for the following 10 candidate substances it can be concluded that they will be metabolised into innocuous metabolites: [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] from subgroup I and [FL-no: 01.001, 01.027, 01.028, 01.039 and 01.046] from subgroup III. For four candidate substances [FL-no: 01.064, 01.070, 01.035 and 01.059], it cannot be assumed that they are metabolised to innocuous metabolites.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is concluded that the 10 candidate substances which are expected to be metabolised to innocuous substances, [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] from subgroup I and [FL-no: 01.001, 01.027, 01.028, 01.039 and 01.046] from subgroup III, would not give rise to safety concerns at their estimated intakes arising from their use as flavouring substances based on the MSDI approach.

For 4(10)-thujene [FL-no: 01.059], one of the substances from subgroup V which are not expected to be metabolised to innocuous substances, a margin of safety could be calculated based upon a NOAEL (222 mg/kg/bw) for the supporting substance ß-caryophyllene [FL-no: 01.007]. Compared to the MSDI of 4(10)-thujene of 14 µg/*capita* per day equal to 0.2 µg/kg bw per day, the NOAEL provides a margin of safety of $9.5 \times 10^5$.

For the three remaining substances, 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070] margins of safety could be calculated based upon a NOAEL (44 mg/kg bw) for the supporting substance myrcene [FL-no: 01.008]. Compared to the MSDI of 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070] of 9.1, 14 and 0.0085 µg/*capita* per day equal to 0.15, 0.23 and 0.00014 µg/kg bw per day, the NOAEL provides margins of safety of $2.9 \times 10^5$, $1.9 \times 10^5$ and $3.1 \times 10^8$.

The mTAMDI values for 11 candidate substances are above the threshold for structural class I of 1800 µg/person per day. For these substances more reliable exposure data are requested in order for them to be considered using the Procedure. For limonene [FL-no: 01.001] the mTAMDI is 1800 µg/person per day and for each of l-limonene and 1-octene [FL-no: 01.046 and 01.070] the mTAMDI is 1600 µg/person per day.

Intake estimates according to the mTAMDI for the 11 candidate substances for which use levels exceed the thresholds of concern for the structural class, and more reliable exposure data are requested. On the basis of such additional data, the flavouring substances should be considered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether this conclusion could be applied to the materials of commerce, it is necessary to consider the available specifications.

Specifications including complete purity criteria and identity for the materials of commerce have been provided for all 14 flavouring substances.

Thus, for all 14 candidate substances [FL-no: 01.001, 01.027, 01.028, 01.033, 01.034, 01.035, 01.038, 01.039, 01.046, 01.054, 01.057, 01.059 and 01.070] the Panel concluded that they would present no safety concern at their estimated levels of intake based on the MSDI approach.

## SUMMARY OF SAFETY EVALUATION

**Table 7:** Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

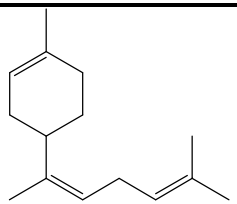
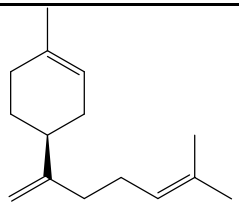
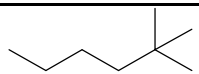
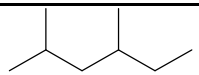
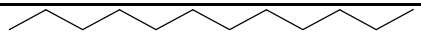
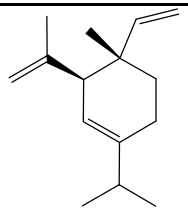
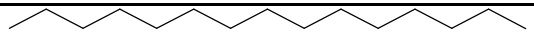
| FL-no | EU Register name | Structural formula | MSDI [(a)] (µg/*capita* per day) | Class [(b)] Evaluation procedure path [(c)] | Outcome on the named compound (d or e) | Outcome on the material of commerce (f, g, or h) | Evaluation remarks |
|---|---|---|---|---|---|---|---|
| 01.027 | Bisabola-1,8,12-triene |  | 0.024 | Class I<br>A3: Intake below threshold | d | g | |
| 01.028 | beta-Bisabolene |  | 2.7 | Class I<br>A3: Intake below threshold | d | f | |
| 01.033 | 2,2-Dimethylhexane |  | 1.2 | Class I<br>A3: Intake below threshold | d | f | |
| 01.034 | 2,4-Dimethylhexane |  | 1.2 | Class I<br>A3: Intake below threshold | d | f | |
| 01.038 | Dodecane |  | 0.012 | Class I<br>A3: Intake below threshold | d | f | |
| 01.039 | delta-Elemene |  | 0.012 | Class I<br>A3: Intake below threshold | d | f | |
| 01.054 | Pentadecane |  | 0.61 | Class I<br>A3: Intake below threshold | d | f | |

**Table 7:** Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

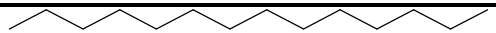
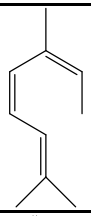
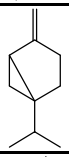
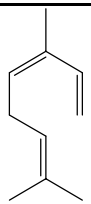
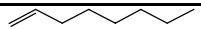
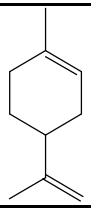
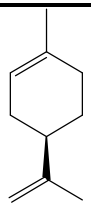
| FL-no | EU Register name | Structural formula | MSDI [(a)] (µg/*capita* per day) | Class [(b)] Evaluation procedure path [(c)] | Outcome on the named compound [(d or e)] | Outcome on the material of commerce [(f, g, or h)] | Evaluation remarks |
|---|---|---|---|---|---|---|---|
| 01.057 | Tetradecane |  | 0.012 | Class I<br>A3: Intake below threshold | d | f | |
| 01.035 | 2,6-Dimethylocta-2,4,6-triene |  | 9.1 | Class I<br>B3: Intake below threshold, B4: No adequate NOAEL | Additional data required | | |
| 01.059 | 4(10)-Thujene |  | 14 | Class I<br>B3: Intake below threshold, B4: Adequate NOAEL exists | d | | |
| 01.064 | cis-3,7-Dimethyl-1,3,6-octatriene |  | 14 | Class I<br>B3: Intake below threshold, B4: No adequate NOAEL | Additional data required | | |
| 01.070 | 1-Octene |  | 0.0085 | Class I<br>B3: Intake below threshold, B4: No adequate NOAEL | Additional data required | | |
| 01.001 | Limonene |  | 4000 | Class I<br>A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists | d | f | |

**Table 7:** Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

| FL-no | EU Register name | Structural formula | MSDI [(a)] (µg/*capita* per day) | Class [(b)] Evaluation procedure path [(c)] | Outcome on the named compound [(d or e)] | Outcome on the material of commerce [(f, g, or h)] | Evaluation remarks |
|---|---|---|---|---|---|---|---|
| 01.046 | l-Limonene |  | 2100 | Class I<br>A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists | d | f | |

(a): EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita per day.
(b): Thresholds of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
(h): No conclusion can be drawn due to lack of information on the purity of the material of commerce.

## SUPPORTING SUBSTANCES SUMMARY

**Table 8:** Supporting Substances Summary

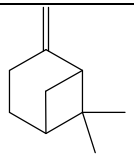
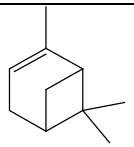
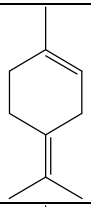
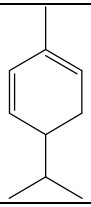
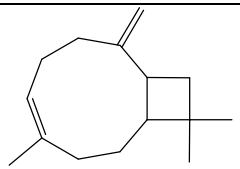
| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | JECFA no<br>Specification available | MSDI (EU) [a]<br>(μg/*capita* per day) | SCF status [b]<br>JECFA status [c]<br>CoE status [d] | Comments |
|---|---|---|---|---|---|---|---|
| 01.003 | Pin-2(10)-ene |  | 2903<br>2114<br>127-91-3 | 1330<br>JECFA specification (JECFA, 2005a). | 1300 | No safety concern (JECFA, 2005b)<br>Category B (CoE, 1992) | JECFA name: beta-Pinene.<br>EFSA conclusion: additional data required (EFSA, 2009b). |
| 01.004 | Pin-2(3)-ene |  | 2902<br>2113<br>80-56-8 | 1329<br>JECFA specification (JECFA, 2005a) | 1800 | No safety concern (JECFA, 2005b)<br>Category B (CoE, 1992) | JECFA name: Alpha-Pinene.<br>EFSA conclusion: additional data required (EFSA, 2009b). |
| 01.005 | Terpinolene |  | 3046<br>2115<br>586-62-9 | 1331<br>JECFA specification (JECFA, 2005a). | 660 | No safety concern (JECFA, 2005b)<br>Category B (CoE, 1992) | |
| 01.006 | alpha-Phellandrene |  | 2856<br>2117<br>99-83-2 | 1328<br>JECFA specification (JECFA, 2005a). | 79 | No safety concern (JECFA, 2005b)<br>Category B (CoE, 1992) | |
| 01.007 | beta-Caryophyllene |  | 2252<br>2118<br>87-44-5 | 1324<br>JECFA specification (JECFA, 2005a). | 330 | No safety concern (JECFA, 2005b)<br>Category B (CoE, 1992) | EFSA conclusion: additional data required (EFSA, 2009b). |

**Table 8:** Supporting Substances Summary

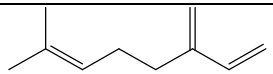
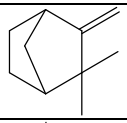
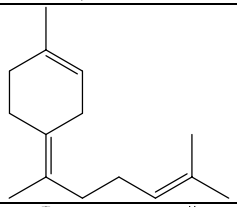
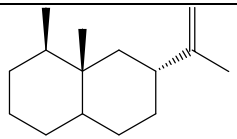
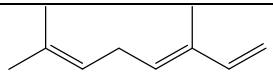
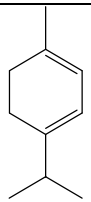
| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | JECFA no<br>Specification available | MSDI (EU) [a]<br>(μg/*capita* per day) | SCF status [b]<br>JECFA status [c]<br>CoE status [d] | Comments |
|---|---|---|---|---|---|---|---|
| 01.008 | Myrcene |  | 2762<br>2197<br>123-35-3 | 1327<br>JECFA specification (JECFA, 2005a) | 290 | No safety concern (JECFA, 2005b)<br>Category B (CoE, 1992) | EFSA conclusion: B4-No, additional data required (EFSA, 2009b). |
| 01.009 | Camphene |  | 2229<br>2227<br>79-92-5 | 1323<br>JECFA specification (JECFA, 2005a) | 13 | No safety concern (JECFA, 2005b)<br>Category B (CoE, 1992) | EFSA conclusion: additional data required (EFSA, 2009b). |
| 01.016 | 1,4(8),12-Bisabolatriene |  | 3331<br>10979<br>495-62-5 | 1336<br>JECFA specification (JECFA, 2005a). | 13 | No safety concern (JECFA, 2005b) | JECFA name: Bisabolene. |
| 01.017 | Valencene |  | 3443<br>11030<br>4630-07-3 | 1337<br>JECFA specification (JECFA, 2005a) | 53 | No safety concern (JECFA, 2005b) | EFSA conclusion: additional data required (EFSA, 2009b). |
| 01.018 | beta-Ocimene |  <br>(E)- isomer shown | 3539<br>11015<br>13877-91-3 | 1338<br>JECFA specification (JECFA, 2005a). | 55 | No safety concern (JECFA, 2005b) | JECFA name: 3,7-Dimethyl-1,3,6-octatriene. |
| 01.019 | alpha-Terpinene |  | 3558<br>11023<br>99-86-5 | 1339<br>JECFA specification (JECFA, 2005a) | 28 | No safety concern (JECFA, 2005b) | JECFA name: p-Mentha-1,3-diene. |

**Table 8:** Supporting Substances Summary

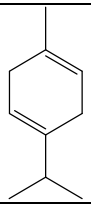
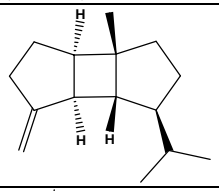
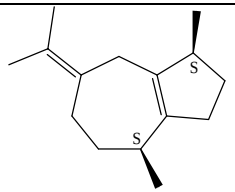
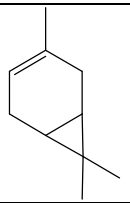
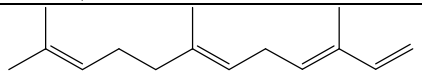
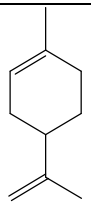
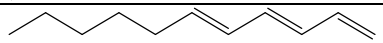
| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | JECFA no<br>Specification available | MSDI (EU) [(a)]<br>(μg/*capita* per day) | SCF status [(b)]<br>JECFA status [(c)]<br>CoE status [(d)] | Comments |
|---|---|---|---|---|---|---|---|
| 01.020 | gamma-Terpinene |  | 3559<br>11025<br>99-85-4 | 1340<br>JECFA specification (JECFA, 2005a) | 1200 | No safety concern (JECFA, 2005b) | JECFA name: p-Mentha-1,4-diene. |
| 01.024 | beta-Bourbonene |  | 11931<br>5208-59-3 | 1345<br>JECFA specification (JECFA, 2005a). | 0.012 | No safety concern (JECFA, 2005b) | EFSA conclusion: additional data required (EFSA, 2009b). MSDI based on USA production figure. |
| 01.026 | 1(5),7(11)-Guaiadiene |  | 88-84-6 | 1347<br>JECFA specification (JECFA, 2005a). | 0.012 | No safety concern (JECFA, 2005b) | JECFA name: Guaiene. EFSA conclusion: additional data required (EFSA, 2009b). |
| 01.029 | delta-3-Carene |  | 3821<br>10983<br>13466-78-9 | 1342<br>JECFA specification (JECFA, 2005a) | 290 | No safety concern (JECFA, 2005b) | EFSA conclusion: additional data required (EFSA, 2009b). |
| 01.040 | alpha-Farnesene |  | 3839<br>10998<br>502-61-4 | 1343<br>JECFA specification (JECFA, 2005a). | 0.61 | No safety concern (JECFA, 2005b) | |

**Table 8:** Supporting Substances Summary

| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | JECFA no<br>Specification available | MSDI (EU) [(a)]<br>(µg/*capita* per day) | SCF status [(b)]<br>JECFA status [(c)]<br>CoE status [(d)] | Comments |
|---|---|---|---|---|---|---|---|
| 01.045 | d-Limonene |  | 2633<br>491<br>5989-27-5 | 1326<br>JECFA specification (JECFA, 2005a). | 34000 | No safety concern (JECFA, 2005b) | ADI not specified (JECFA, 2006). |
| 01.061 | Undeca-1,3,5-triene |  | 3795<br><br>16356-11-9 | 1341<br>JECFA specification (JECFA, 2005a). | 0.24 | No safety concern (JECFA, 2005b) | JECFA name: 1,3,5-Undecatriene. EFSA conclusion: additional data required (EFSA, 2009b). |

(a): EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita per day.
(b): Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.
(c): No safety concern at estimated levels of intake.
(d): Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

## TOXICITY DATA

**Table 9:** Acute Toxicity

| Chemical Name [FL-no] * | Species | Sex | Route | $LD_{50}$ (mg/kg bw) | Reference | Comments |
|---|---|---|---|---|---|---|
| Tetradecane [01.057] | Rat | NR | Oral | > 5000 | (Enichem Augusta Ind., 1987) | Study not available |
| | Rat | NR | Oral | > 5000 | (PETRESA, 19??b) | Study not available |
| (Undeca-1,3,5-triene [01.061]) | Rat | M, F | Oral | > 8000 | (Pellmont, 1973) | Data not possible to interprete |
| | Mouse | M, F | Oral | 2000 - 4000 | (Pellmont, 1973) | Data not possible to interprete |
| (Myrcene [01.008]) | Rat | M | Oral | > 5000 | (Moreno, 1972a) | Identity of compound not stated as other than code number |
| (β-Ocimene [01.018]) | Rat | NR | Oral | 5000 | (Moreno, 1976a) | One dose tested |
| (*d*-Limonene [01.045]) | Rat | M | Oral | > 5000 | (Moreno, 1972d) | Identity of compound not stated as other than code number |
| | Rat | M, F | Oral | M: 4400<br>F: 5100 | (Tsuji et al., 1975a) | Study is on dogs, rats not mentioned in English text. Study in Japanese |
| | Mouse | M, F | Oral | M: 5600<br>F: 6600 | (Tsuji et al., 1975a) | Study is on dogs, mice not mentioned in English text. Study in Japanese |
| (Terpinolene [01.005]) | Rat | NR | Oral | 4.39 ml/kg (3784) | (Levenstein, 1975) | Observation period not given. |
| (α-Terpinene [01.019]) | Rat | NR | Oral | 1680 | (Moreno, 1973b) | |
| (gamma-Terpinene [01.020]) | Rat | M, F | Oral | 3650 | (Moreno, 1973a) | |
| (α-Phellandrene [01.006]) | Rat | M, F | Oral | > 5700 | (Moreno, 1972b) | Submitted study is on dermal toxicity. |
| | Rat | M, F | Oral | 1.87 ml/kg (1590) | (Brownlee, 1940) | Substance mixed with acacia. 6 animals per dose group |
| β-Bisabolene [01.028] | Rat | NR | Oral | > 5000 | (Moreno, 1974a) | One dose tested |
| | Mouse | M, F | Oral | > 13,360 | (Hoffman-LaRoche, Inc., 1967) | No of dose levels not given. |
| (delta-3-Carene [01.029]) | Rat | M | Oral | 4800 | (Moreno, 1972c) | Identity of compound not stated |
| (Pin-2(3)-ene [01.004]) | Rat | M, F | Oral | 3700 | (Moreno, 1972e) | Identity of compound not stated |
| (Pin-2(10)-ene [01.003]) | Rat | NR | Oral | > 5000 | (Moreno, 1975) | One dose tested. |
| (Camphene [01.009]) | Rat | NR | Oral | > 5000 | (Moreno, 1974b) | One dose tested |
| (Valencene [01.017]) | Rat | M | Oral | > 5000 | (Moreno, 1980) | One dose tested |
| (β–Caryophyllene [01.007]) | Rat | M, F | Oral | > 5000 | (Hart and Wong, 1971) | One dose tested |
| (1(5),7(11)-Guaiadiene [01.026]) | Rat | NR | Oral | > 5000 | (Moreno, 1976b) | One dose tested |
| (1-methyl cyclohexa-1,3-diene [01.077]) | Rat | M, F | Oral | > 2000 | (Felice, 2005) | Acute toxic class method. 2000 mg/kg is only dose tested, no death was observed. |

* Supporting substances are listed in brackets.

**Table 10:** Subacute / Subchronic / Chronic / Carcinogenicity Studies

| Chemical Name [FL-no] | Species; Sex No./Group | Route | Dose levels | Duration | NOAEL (mg/kg bw per day) | Reference | Comments |
|---|---|---|---|---|---|---|---|
| (Undeca-1,3,5-triene [01.061]) | Rat; M, F 10 | Diet | Calculated to provide 10 mg Galbelica/kg bw per day corresponding to 2 mg undecatriene, 8 mg pinene and the rest dodecene. | 14 days | Corresponding to 2 mg undecatriene[1] | (Shapiro, 1988) | Not valid. See footnote[1]. |
| (*d*-Limonene [01.045]) | Rat; M 5 | Gavage | 0, 75, 150 or 300 mg/kg bw per day 5 days a week | 6 or 27 days (5 or 25 doses) | 75 | (Kanerva et al., 1987) | Study is on kidney toxicity specifically. |
| | Rat; M 5 – 10 | Gavage | 0, 2, 5, 10, 30 or 75 mg/kg bw per day, 5 days/week | 90 days | 5 | (Webb et al., 1989) | Study is on kidney toxicity specifically. |
| | Dog; M, F 6 | Gavage | 0, 0.4, 1.2 or 3.6 ml/kg bw per day corresponding to 0, 340, 1000 or 3000 mg/kg bw per day | 180 days | M: 340<br>F: 340 | (Tsuji et al., 1975a) | Study is in Japanese, only summary and tables in English. Quality of study not possible to assess. |
| | Dog; M, F 10 | Gavage (divided doses) | 0, 0.12 or 1.2 ml/kg bw per day corresponding to 0, 100 or 1000 mg/kg bw per day. | 180 days | 100 | (Webb et al., 1990) | |
| | Mouse; M, F 10 | Gavage | 0, 413, 825, 1650, 3300 or 6600 mg/kg bw per day for 12 days over 16-day period | 16 days | 1650 | (NTP, 1990) | |
| | Mouse; M, F 20 | Gavage | 0, 125, 250, 500, 1000 or 2000 mg/kg bw per day 5 days per week | 90 days | M: 500<br>F: 500 | (NTP, 1990) | Clinical signs of rough hair coat and decrased activity were observed at the two highest doses. |
| | Mouse; M, F M:50 F:50 | Gavage | M: 0, 250 or 500 mg/kg bw per day<br>F: 0, 500, 1000 mg/kg bw per day<br>5 days per week | 2 years | M: 250<br>F: 500 | (NTP, 1990) | |
| | Rat; M, F 10 | Gavage | 0, 413, 825, 1650, 3300 or 6600 mg/kg bw per day for 12 days over 16-day period | 16 days | 1650 | (NTP, 1990) | |
| | Rat; M, F 20 | Gavage | 0, 150, 300, 600, 1200 or 2400 mg/kg bw per day 5 days per week | 90 days | M: None<br>F: 1200 | (NTP, 1990) | Dose-dependent nephropathy in all treated males. |

**Table 10:** Subacute / Subchronic / Chronic / Carcinogenicity Studies

| Chemical Name [FL-no] | Species; Sex No./Group | Route | Dose levels | Duration | NOAEL (mg/kg bw per day) | Reference | Comments |
|---|---|---|---|---|---|---|---|
| | Rat; M, F M:50 F:50 | Gavage | M: 0, 75 or 150 mg/kg bw per day F: 0, 300 or 600 mg/kg bw per day 5 days per week | 2 years | M: None F: 300 | (NTP, 1990) | |
| (Pin-2(10)-ene [01.003]) | Rat; M, F 10 | Diet | Calculated to provide 10 mg Galbelica/kg bw per day corresponding to 8 mg pin-2(10)-ene | 14 days | Corresponding to 8 mg pin-2(10)-ene[1] | (Shapiro, 1988) | Not valid. See footnote[1]. |
| (Camphene [01.009]) | Rat; M, F 10 | Gavage | 0, 62.5, 250, 1000 mg/ kg bw per day | 28 days | M: None F: 250 | (Hoechst, 1991) | Study report very limited in details. Study according to OECD Guideline 407. |
| (Myrcene [01.008]) | Mouse; M,F M:10 F:10 | Gavage | 0, 250, 500, 1000, 2000 or 4000 mg/kg bw for 5 days a week | 90 days | 250 | (NTP, 2010) | This study was performed at a either a single dose or multiple dose levels that produced no effects. Therefore, this dose level is not a true NOEL, but the highest dose tested that produced no adverse effects. The actual NOEL would be higher. |
| | Mouse; M, F M:50 F:50 | Gavage | 0, 250, 500 or 1000 mg/kg bw for 5 days a week | 2 years | 250 | (NTP, 2010) | |
| | Rat; M, F M:10 F:10 | Gavage | 0.250, 500, 1000, 2000 or 4000 mg/kg bw per day | 90 days | | (NTP, 2010) | Study showed nephrotoxicity at the lowest dose level (250 mg/kg body wt) |
| | Rat; M, F M:50 F:50 | Gavage | 0 ,250, 500 or 1000 mg/kg bw for 5 days a week | 2 years | | (NTP, 2010) | Study showed nephrotoxicity at the lowest dose level and kidnet tumors at hihger dose levels |
| | Rat; M, F M:10 F:10 | Diet | 0, 8,0 , 40 , and 44 mg/kg bw per day (males) 0, 9,6 , 48 , and 53 mg/kg bw per day (females) | 90 days | 44 (males) 53 (females) | (Bauter, 2013b) | No toxicity at highest dose level |

**Table 10:** Subacute / Subchronic / Chronic / Carcinogenicity Studies

| Chemical Name [FL-no] | Species; Sex No./Group | Route | Dose levels | Duration | NOAEL (mg/kg bw per day) | Reference | Comments |
|---|---|---|---|---|---|---|---|
| (β-Caryophyllene [01.007]) | Rat; M, F M:10 F:10 | Diet | 0, 220, 460 and 1.370 mg/kg bw per day (males)<br>0, 260, 1.030 and 4.280 mg/kg bw per day (females), | 90 days | 222 (males)<br>263(females) | (Bauter, 2013a) | Study according to OECD Guideline 408. |
| | Rat; M, F M:3 F:3 | Diet | 0, 520, 1.550 and 3.570mg/kg bw per day (males)<br>0, 530, 1.580 and 4.440 mg/kg bw per day (females) | 14 days | - | (Bauter, 2011) | |

[1] Study performed using a dose of 10 mg/kg body weight per day of Galbelica, which is a solution composed of 80 % β-pinene and 20 % 1,3,5-undecatriene and dodecene.

**Table 11:** Developmental and Reproductive Toxicity Studies

| Chemical Name [FL-no] | Study type Durations | Species/Sex No / group | Route | Dose levels | NOAEL (mg/kg bw per day), Including information of possible maternal toxicity | Reference | Comments |
|---|---|---|---|---|---|---|---|
| (Myrcene [01.008]) | Developmental Toxicity: Gestation Days 6 - 15 | Rat; F 16 - 29 | Gavage | 250, 500 or 1200 mg/kg bw per day | Maternal: 500[1] Foetal: 500 | (Delgado et al., 1993a) | Study considered valid. |
| | Peri- and Postnatal Developmental Toxicity: Gestation Day 15 to Postnatal Day 21 | Rat; F 12 - 18 | Gavage | 0, 250, 500, 1000, 1500 mg/kg bw per day | Maternal: 500[2] Peri- and Post-natal: 250 | (Delgado et al., 1993b) | Study considered valid. |
| | Reproductive and developmental toxicity: Prior to mating until postnatal day 21 | Rat; M, F 60 | Gavage | 0, 100, 300, 500 mg/kg bw per day | Maternal/paternal: 500 mg/kg bw per day Foetal: 300 mg/kg bw per day | (Paumgartten et al., 1998) | Study considered valid. |
| (d-Limonene [01.045]) | Developmental Toxicity: Gestation Days 9 - 15 | Rat; F 20 | Oral | 0, 591 or 2869 mg/kg bw per day | Maternal: 591 Foetal: 591 | (Tsuji et al., 1975b) | Study in Japanese, only summary and tables in English. Quality of study not possible to assess. |
| | Developmental Toxicity: Gestation Days 7 - 12 | Mouse; F 15 | Oral | 0, 591 or 2363 mg/kg bw per day | Maternal: 591 Foetal: 591 | (Kodama et al., 1977a) | Study in Japanese, only summary and tables in English. Quality of study not possible to assess. |
| | Developmental toxicity: gestation days 6 - 18 | Rabbit; F 10, 18 in highest dose group | Oral | 0, 250, 500 or 1000 mg/kg bw per day | Maternal: 250 Foetal: 1000 | (Kodama et al., 1977b) | Study in Japanese, only summary and tables in English. Quality of study not possible to assess. |
| (Pin-2(3)-ene [01.004]) | Developmental toxicity: Gestation days 9 - 14 | Rat; F 12 - 17 | Oral | Not relevant | Not relevant | (Hasegawa and Toda, 1978) | Study is not considered valid. Study is on a mixture of menthol, menthone, pinene, borneol cineol, camphene rheochrysin in olive oil. Pin-2(3)-ene content is 17 %. Study in Japanese. |
| | Developmental toxicity: Gestation Days 6 - 15 | Rat; F 20 | Gavage | | Maternal: 250[3] Foetal: 1000 | (Leuschner, 1992; LPT Research, 1992) | Study not submitted. |

1 Test substance was myrcene.
2 Study performed using a dose of 10 mg/kg body weight per day of Galbelica, which is a solution composed of 80 % β-pinene and 20 % 1,3,5-undecatriene.
3 Test substance was 78 % pure.

**Table 12:** Genotoxicity (*in vitro*)

| Chemical Name [FL-no] * | Test System | Test Object | Concentration | Result | Reference | Comments |
|---|---|---|---|---|---|---|
| Cedrene washed[6] [CAS no 11028-42-5] | Ames test | *S. typhimurium* TA97, TA98; TA100; TA1535; TA102 | 8-5000[4] | Negative[1] | (Gocke, 1999) | Validity cannot be evaluated as substance is not specified.<br>Cedarwood oil terpenes and tertonoids. |
| | Ames test | *S. typhimurium* TA97, TA98; TA100; TA1535; TA102 | 1.6-1000[5] | Negative[1] | (Gocke, 1999) | Validity cannot be evaluated as substance is not specified.<br>Cedarwood oil terpenes and tertonoids. |
| Dodecane [01.038] | Ames test | *S. typhimurium* TA98; TA100 | NR | Negative[1] | (Tummey et al., 1992) | Only part of abstract available. Validity of the study cannot be evaluated due to insufficient report of experimental details and results. |
| | Mammalian cell gene mutation test (mouse lymphoma assay) | Mouse lymphocytes | NR | Negative[1] | (Tummey et al., 1992) | Only part of abstract available. Validity of the study cannot be evaluated due to insufficient report of experimental details and results. |
| | Mammalian cell gene mutation test | V79 Chinese hamster ovary cells | 0.12 mM (20 µg/ml) | Negative[3] | (Lankas et al., 1978) | Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Study designed to evaluate the ability of various alkanes to enhance the mutagenicity induced by the chemical carcinogen methylazoxymethanol acetate. Dodecane showed no mutagenic activity per se, but increased the mutagenesis induced by pretreatment with the carcinogen. |
| Tetradecane [01.057] | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 | 50, 150, 500, 1500, 5000 µg/plate | Negative[1] | (PETRESA, 19??a) | (Study carried out by Huntingdon Research Centre, Report PEQ 5C/85914, sponsored by PETRESA; year not indicated)<br>Unpublished GLP-study carried out in accordance with OECD guideline 471 as stated in the IUCLID datasheet submitted. IUCLID abstract available only. Validity of the study cannot be evaluated. |

**Table 12:** Genotoxicity (*in vitro*)

| Chemical Name [FL-no] * | Test System | Test Object | Concentration | Result | Reference | Comments |
|---|---|---|---|---|---|---|
| | Mammalian cell gene mutation test | V79 Chinese hamster ovary cells | 0.12 mM (23 µg/ml) | Negative[3] | (Lankas et al., 1978) | Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Study designed to evaluate the ability of various alkanes to enhance the mutagenicity induced by the chemical carcinogen methylazoxymethanol acetate. Tetradecane showed no mutagenic activity per se, but increased the mutagenesis induced by pretreatment with the carcinogen. |
| | Ames test (preincubation method) | *S. typhimurium* TA98; TA100; UTH8414; UTH8413 | 0, 50, 100, 500, 1000, 2000 µg/plate | Negative[1] | (Connor et al., 1985) | Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated. Cytotoxicity not reported. |
| (2-Methylbuta-1,3-diene) or isoprene | Ames test | *S. typhimurium* TA98; TA100; TA1530; TA1535; TA1538 | 25 % atmosphere concentration | Negative[1] | (De Meester et al., 1981) | Published non-GLP study not in accordance with OECD guideline 471. Part of a larger study evaluating the effects of various experimental conditions (different liver cell preparations and concentrations) on the mutagenic activity of butadiene, hexachlorobutadiene and isoprene. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Plates were exposed to a 25 % 2-methylbuta-1,3-diene atmosphere for 24 hours. |
| | Ames test (preincubation method) | *S. typhimurium* TA98; TA100; TA1535; TA1537 | 0, 100, 333, 1000, 3333, 10000 µg/plate | Negative[1] | (Mortelmans et al., 1986) (NTP, 1999) | Published summary report including detailed results from studies on 270 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471. |
| | Ames test | *S. typhimurium* TA102; TA104 | NR | Negative | (Kushi et al., 1985) | Published abstract only, of which part of the text including results is missing. No information on the use of a metabolic activation system. Validity of the study cannot be evaluated. |
| | Ames test (preincubation method) | *S. typhimurium* TA98; TA100; TA1535 *E. coli* WP2uvrA/pKM101 | 0, 500, 1000, 2000, 5000 µg/plate | Negative[1] | (Madhusree et al., 2002) | Published non-GLP study with limited report of experimental details and results. Thus, the validity of the study cannot be evaluated. |

**Table 12:** Genotoxicity (*in vitro*)

| Chemical Name [FL-no] * | Test System | Test Object | Concentration | Result | Reference | Comments |
|---|---|---|---|---|---|---|
| | Sister chromatid exchange test | Chinese hamster ovary cells | 0, 50, 160, 500, 1600 µg/ml (-S9)<br>0, 160, 500, 1600, 5000 µg/ml (+S9). | Negative[1] | (NTP, 1999; Galloway et al., 1987) | Published summary report including detailed results from studies on 108 chemicals tested within the NTP to a large extent in accordance with OECD guideline 479. |
| | Chromosomal aberration assay | Chinese hamster ovary cells | 0, 1600, 3000, 5000 µg/ml | Negative[1] | (NTP, 1999; Galloway et al., 1987) | Published summary report including detailed results from studies on 108 chemicals tested within the NTP to a large extent in accordance with OECD guideline 473. |
| (Myrcene [01.008]) | Chromosomal aberration assay | Human lymphocytes | 1000 µg/ml | Negative[1] | (Kauderer et al., 1991) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Mammalian cell gene mutation assay | Chinese hamster ovary V79 cells | 1000 µg/ml | Negative[1] | (Kauderer et al., 1991) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Sister chromatid exchange test | Human lymphocytes | 1000 µg/ml | Negative[1] | (Kauderer et al., 1991) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Sister chromatid exchange test | Chinese hamster ovary cells and hepatic tumour cell line | 500 µg/ml | Negative[1] | (Röscheisen et al., 1991) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test (plate incorporation method) | *S. typhimurium* TA97a ; TA98; TA100; TA1535 | Up to 1500 µg/plate (16 concentrations) | Negative | (Gomes-Carneiro et al., 2005) | Valid studies which were carried out with a selection of 6 of the the concentrations mentioned. In the first run concentrations up to cytotoxicity were studied; in a second run only non-toxic concentrations were tested. |
| | Ames | *S. typhimurium* TA97; TA98; TA100; TA1535 | 10 – 10 000 µg/plate | Negative[1] | (NTP, 2010) | |
| | Reverse mutation | *E. coli* WP2*uvrA*/pKM101 | 50 – 10 000 µg/plate | Negative[1] | (NTP, 2010) | |
| | Ames | *S. typhimurium* TA97a; TA98; TA100; TA1535 | 10 - 5000 | Negative[1] | (Gomes-Carneiro et al., 2005) | |
| | Ames | *S. typhimurium* TA97a; TA98; TA100; TA1535 | 1 - 1500 | Negative[1] | (Gomes-Carneiro et al., 2005) | |

**Table 12:** Genotoxicity (*in vitro*)

| Chemical Name [FL-no] * | Test System | Test Object | Concentration | Result | Reference | Comments |
|---|---|---|---|---|---|---|
| (*d*-Limonene [01.045]) | Ames test (preincubation method) | *S. typhimurium* TA98; TA100; TA1535; TA1537 | 0.03, 0.3, 3, 30 µM/plate (4.1, 41, 410, 4100 µg/plate) | Negative[1] | (Florin et al., 1980) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 | Up to 150,000 µg/plate | Negative[1] | (Heck et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test | *S. typhimurium* TA102 | Up to 5000 µg/plate | Negative[1] | (Müller et al., 1993) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537 | Up to 3333 µg/plate | Negative[1] | (Haworth et al., 1983) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test (preincubation method) | *S. typhimurium* TA98; TA100, UTH8413 and UTH8414 | 0, 10 to 500 µg/plate (5 concentrations) | Negative[1] | (Connor et al., 1985) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Forward mutation assay | L5178Y Mouse lymphoma | Up to 100 µg/ml | Negative[1] | (Heck et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Forward mutation assay | L5178Y Mouse Lymphoma | Up to 100 µg/ml | Negative[1] | (Myhr et al., 1990) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Chromosomal aberration assay | Chinese hamster ovary cells | 500 µg/ml | Negative[1] | (Anderson et al., 1990) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Sister chromatid exchange test | Chinese hamster ovary cells | 162 µg/ml | Negative[1] | (Anderson et al., 1990) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Sister chromatid exchange test | Chinese hamster ovary cells | 1000 µM (136.2 µg/ml) | Negative | (Sasaki et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| (gamma-Terpinene [01.020]) | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 | 50,000 µg/plate | Negative[1] | (Heck et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Unscheduled DNA synthesis | Rat hepatocytes | 30 µg/ml | Negative | (Heck et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| (α-Terpinene [01.019]) | Ames test (plate incorporation method) | *S. typhimurium* TA97a ; TA98; TA100; TA1535 | Up to 1500 µg/plate (13 concentrations) | Negative | (Gomes-Carneiro et al., 2005) | Valid studies which were carried out with a selection of 6 of the the concentrations mentioned. In the first run concentrations up to cytotoxicity were studied; in a second run only non-toxic concentrations were tested. |

**Table 12:** Genotoxicity (*in vitro*)

| Chemical Name [FL-no] * | Test System | Test Object | Concentration | Result | Reference | Comments |
|---|---|---|---|---|---|---|
| (α-Phellandrene [01.006]) | Sister chromatid exchange test | Chinese hamster ovary cells | 1000 µM(136.2 µg/ml) | Negative | (Sasaki et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| (delta-3-Carene [01.029]) | Ames test (plate incorporation method) | *S. typhimurium* TA98; TA100; TA102 | up to 5 µl/plate (up to 4300 µg/plate; 5 concentrations) | Positive[3] Negative[2] | (Kurttio et al., 1990) | Published non-GLP study with insufficiently reported results. Limited validity. Positive without metabolic activation in TA100 and TA102 and at doses of 2.5 µl/plate and higher. |
| (Pin-2(3)-ene [01.004]) | Ames test | *S. typhimurium* TA98; TA100 | 100 µl/plate (85,800 µg/ plate) | Negative[2] | (Rockwell and Raw, 1979) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537 | 0.03, 0.3, 3, 30 µM/plate (4.1, 41, 410, 4100 µg/ plate) | Negative[1] | (Florin et al., 1980) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 | 25000 µg/plate | Negative[1] | (Heck et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 | 25 µl/plate (21,450 µg/ plate) | Negative[1] | (Jagannath, 1984) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 | 5 µl/plate (4290 µg/plate) | Negative[1] | (DeGraff, 1983) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test | *S. typhimurium* TA98; TA100; UTH8414; UTH8413 | 0, 10 to 500 µg/plate (5 concentrations) | Negative[1] | (Connor et al., 1985) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Unscheduled DNA synthesis | Rat hepatocytes | 10000 µg/ml | Negative | (Heck et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| (+)-α-pinene (pin-2(3)-ene) (isomer of [01.004]) | Ames test (plate incorporation method) | *S. typhimurium* TA97a ; TA98; TA100; TA1535 | Up to 1000 µg/plate (18 concentrations) | Negative | (Gomes-Carneiro et al., 2005) | Valid studies. |
| (-)-α-pinene (pin-2(3)-ene) (isomer of [01.004]) | Ames test (plate incorporation method) | *S. typhimurium* TA97a ; TA98; TA100; TA1535 | Up to 4000 µg/plate (19 concentrations) | Negative | (Gomes-Carneiro et al., 2005) | Valid studies. |

**Table 12:** Genotoxicity (*in vitro*)

| Chemical Name [FL-no] * | Test System | Test Object | Concentration | Result | Reference | Comments |
|---|---|---|---|---|---|---|
| (Pin-2(10)-ene [01.003]) | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 | 5000 µg/plate | Negative[1] | (Heck et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test (preincubation method) | *S. typhimurium* TA98; TA100; TA1535; TA1537 | 0.03, 0.3, 3, 30 µM/plate (4.1, 41, 410, 4100 µg/plate) | Negative[1] | (Florin et al., 1980) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Sister chromatid exchange | Chinese hamster ovary cells | 1000 µM (136.2 µg/ml) | Negative | (Sasaki et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| (Camphene [01.009]) | Ames test | *S. typhimurium* TA98; TA100 | 100 µl/plate (84,500 µg/ plate) | Negative[2] | (Rockwell and Raw, 1979) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test | *S. typhimurium* TA98; TA100; UTH8414; UTH8413 | 0, 10 to 1000 µg/plate (5 concentrations) | Negative[1] | (Connor et al., 1985) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Sister chromatid exchange test | Chinese hamster ovary cells | 1000 µM (136.2 µg/ml) | Negative | (Sasaki et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| (β–Caryophyllene [01.007]) | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 | 150,000 µg/plate | Negative[1] | (Heck et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test | *S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 | 150 µl/plate | Negative[1] | (Lorillard, 1984) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Ames test (plate incorporation method) | *S. typhimurium* TA98; TA100; TA102; TA1535; TA1537 | 10,000 µg/plate | Negative[1] | (Longfellow, 1998) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Sister chromatid exchange test | Chinese hamster ovary cells | 1000 µM (204.4 µg/ml) | Negative | (Sasaki et al., 1989) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |

**Table 12:** Genotoxicity (*in vitro*)

| Chemical Name [FL-no] * | Test System | Test Object | Concentration | Result | Reference | Comments |
|---|---|---|---|---|---|---|
| | Ames test (plate incorporation method) | *S. typhimurium* strains TA98; TA100 *E. coli* WP2uvrA | 2300 to 9000 µg/plate | Negative[1] | (Di Sotto et al., 2008) | Study not in compliance with OECD 471: Assay does not include TA 1535 and TA 1537 (or TA97, 97a, resp) - 5 concentrations tested (plate-incorpration) but not given in detail (authors stated that in range finder up to 9 mg/plate without cytotoxicity) results not given in detail (no values, no raw data). Methods and results poorly reported: no historical contral data. The study is considered to be of insufficient quality. |

* Supporting substances are listed in brackets.
NR: Not Reported.
[1] With and without S9 metabolic activation.
[2] With metabolic activation.
[3] Without metabolic activation.
[4] Plate incorporation.
[5] Pre-incubation.
[6] An Ames test with cedrene washed (unspecified cedrene) was also submitted, but an adequate identification of the substance studied was not possible. Therefore the study is not further discussed.

**Table 13:** Genotoxicity (*in vivo*)

| Chemical Name [FL-no] * | Test System | Test Object | Route | Dose | Result | Reference | Comments |
|---|---|---|---|---|---|---|---|
| (2-Methylbuta-1,3-diene) | *In vivo* Chromosomal aberration assay | Mouse (B6C3F1) bone marrow (male mice) | Inhalation | 0, 438, 1750, 7000 ppm for 6 hours per day for 12 exposures over a period of 16 days (Trial 1)<br><br>0, 70, 220, 700 ppm for 6 hours per day for 12 exposures over a period of 16 days (Trial 2) | Negative | (Tice et al., 1987; Tice, 1988; Shelby, 1990) | Unpublished study report and published summary report of a valid multiple endpoint cytogenicity study sponsered by NTP, roughly in accordance with OECD guideline 475 (special dosage regimen used). |
| | *In vivo* Sister chromatid exchange test | Mouse (B6C3F1) bone marrow (male mice) | Inhalation | 0, 438, 1750, 7000 ppm for 6 hours per day for 12 exposures over a period of 16 days (Trial 1)<br><br>0, 70, 220, 700 ppm for 6 hours per day for 12 exposures over a period of 16 days (Trial 2) | Positive | (Tice et al., 1987; Tice, 1988; Shelby, 1990) | Unpublished study report and published summary report of valid cytogenicity study sponsered by NTP. The study is considered valid.<br>Significant ($0.01<p<0.05$) increase in the frequency of SCE in the bone marrow cells at all concentrations. In addition, a significant delay in bone marrow cellular proliferation kinetics (lengthening of the generation time) was detected. The mitotic index was not significantly altered. |
| | *In vivo* Micronucleus test | Mouse (B6C3F1) peripheral blood cells (male mice) | Inhalation | 0, 438, 1750, 7000 ppm for 6 hours per day for 12 exposures over a period of 16 days | Positive | (Tice et al., 1987; Tice, 1988) | Unpublished study report and published summary report of valid cytogenicity study sponsored by NTP, roughly in accordance with OECD guideline 474 (special dosage regimen used). The study is considered valid.<br>Significant ($p<0.001$) increase in the frequency of micronucleated polychromatic and normochromatic erythrocytes, and percentage of PCE. A significant ($p<0.001$) and dose-dependent decrease in the percentage of circulating polychromatic erythrocytes (suppression of erythropoiesis) was noted. |

**Table 13:** Genotoxicity (*in vivo*)

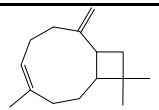
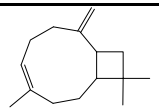
| Chemical Name [FL-no] * | Test System | Test Object | Route | Dose | Result | Reference | Comments |
|---|---|---|---|---|---|---|---|
| | *In vivo* Micronucleus test | Mouse(B6C3F1) peripheral blood cells (male and female mice) | Inhalation | 0, 70, 220, 700, 2200, 7000 ppm for 13-weeks | Positive | (Jauhar et al., 1988) | Study carried out within NTP. Only short summary and tabulated results available from NTP TR 486 (NTP, 1999). Percentage of polychromatic erythrocytes among erythrocytes in peripheral blood was increased probable due to a possible adaptation to cytotoxicity with chronic exposure. Due to the limited details available on this study, the validity cannot be evaluated. |
| | *In vivo* Micronucleus test | Rat lung fibroblasts (male and female rats) | Inhalation | 0, 220, 700, 7000 ppm for 13-weeks | Negative | (Khan and Heddle, 1991) | Study carried out within NTP. Only tabulated results available from NTP TR 486 (NTP, 1999). Unusual study protocol. Validity of the study cannot be evaluated. |
| (Myrcene [01.008]) | *In vivo* Chromosomal aberration assay | Rat (Wistar) bone marrow | Gavage | 0, 100, 500, 1000 mg/kg bw (single exposure) | Negative | (Zamith et al., 1993) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | *In vivo* Micronucleus test | Mouse (B6C3F1) peripheral blood cells | Gavage | 0, 250, 500, 1000, 2000 mg/kg bw (single exposure) | Negative | (NTP, 2003) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | Micronucleus assay | Mouse peripheral blood cells | Gavage | 250, 500, 1000 mg/kg bw/ day | Negative | (NTP, 2010) | |
| (*d*-Limonene [01.045]) | *In vivo* Comet assay | Mouse (ddY) / Rat (Wistar). | Oral | 0, 2000 mg/kg | Negative | (Sekihashi et al., 2002) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | *In vivo* Mammalian spot test | Mouse embryos from C57BL/6JHan x T stocks | Intraperitoneal injection | 215 mg/kg bw | Negative | (Fahrig, 1984) | Abstracted by JECFA at their 63rd meeting (JECFA, 2006). |
| | *In vivo* Comet assay | Rats (Sprague-Dawley) (males) (Kidneys) | Gavage | 0, 1000, 2000 mg/kg bw (single exposure) | Negative | (Nesslany et al., 2007) | |

**Table 13:** Genotoxicity (*in vivo*)

| Chemical Name [FL-no] * | Test System | Test Object | Route | Dose | Result | Reference | Comments |
|---|---|---|---|---|---|---|---|
| | *In vivo* transgenic mutagenisity assay | Rats (Big blue) (males) (liver, kidney, bladder) | Diet | 0, 525 mg/kg bw per day (10 days) | Negative | (Turner et al., 2001) | The author do not specify whether the tested compound is *d*- or *l*-limonene, and the purity of the compound is not stated. However, the stability of the limonene in the diet was measured. |
| (β-caryophyllene [01.007]) | micronucleus induction assay | Mouse (National Institute of Hygiene (NIH), Mexico) | Gavage | 0, 20, 200 and 2000 mg/kg bw | Negative | (Molina-Jasso et al., 2009) | The study is compliance with OCED guideline except: number of micronucleated immature erythrocytes are not given separately for each animal and historical controls not given.<br>The study is reliable with restrictions and therefore, considered to be of limited validity. |

* Supporting substances are listed in brackets.

**Table 14:** Genotoxicity Data of β-Caryophyllene Submitted by EFFA (EFFA, 2012)

| FL-no<br>JECFA -no | EU Register name<br>JECFA name | Structural formula | End-point | Test system | Concentration | Results | Reference | Comments |
|---|---|---|---|---|---|---|---|---|
| ***In vitro*** | | | | | | | | |
| 01.007<br>1324 | β-Caryophyllene |  | Reverse mutation | *S. typhimurium* TA98, TA100, *E. coli* WP2uvrA | 2300–9000 µg/plate | Negative[1] | (Di Sotto et al., 2008) | Study not in compliance with OECD 471:<br>- assay does not include TA1535 and TA1537 (or TA97, 97a, resp)<br>-5 concentrations tested (plate-incorpration) but not given in detail (authors stated that in range finder up to 9 mg/plate without cytotoxicity)<br>-results not given in detail (no values, no raw data).<br>Methods and results poorly reported<br>-no historical contral data<br>insufficient quality. |
| ***In vivo*** | | | | | | | | |
| 01.007<br>1324 | β-Caryophyllene |  | Micronucleated polychromatic erythrocytes | Mice | 0, 20, 200 and 2000 mg/kg bw[2] | Negative | (Molina-Jasso et al., 2009) | In compliance with OECD 474<br>-except: number of micronucleated immature erythrocytes not given separately for each animal; historical controls not given.<br>Reliable with restrictions=limited validity. |

## DOCUMENTATION PROVIDED TO EFSA


1. Bauter MR, 2011. beta-Caryophyllene: palatability/toxicity study: a 14-day dietary study in rats. Product Safety Labs. Study no. 31085. November 16, 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

2. Bauter MR, 2013a. beta-Caryophyllene: a 90-day dietary study in rats. Product Safety Labs. Study no. 33328. January 7, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

3. Bauter MR, 2013b. Myrcene: a 90-day dietary study in rats. Product Safety Labs. Study no. 33546. May 20, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

4. DG SANCO (Directorate General for Health and Consumer Affairs), 2012. Information from DG SANCO 07/02 2012, concerning two lists of 85 and 15 non-supported substances and one list of 30 substances for which no data have been submitted or which are duplicates. FLAVIS.2.23rev1.

5. DG SANCO (Directorate General for Health and Consumer Affairs), 2013. Information from DG SANCO 30/10 2013, concerning a list of 19 non-supported substances. FLAVIS.2.27.

6. DG SANTE (Directorate General for Health and Food safety), 2015. Information from DG SANTE 04 March 2015, concerning the status of substance [FL-no: 01.014].

7. Di Sotto A, Evandri MG and Mazzanti G, 2008. Antimutagenic and mutagenic activities of some terpenes in the bacterial reverse mutation assay. Mutation Research 653, 130-133.

8. EFFA (European Flavour and Fragrance Association), 2002. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.

9. EFFA (European Flavour and Fragrance Association), 2004. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.

10. EFFA (European Flavour and Fragrance Association), 2005a. Submission 2004-3. Flavouring group evaluation of 32 flavouring substances (candidate chemicals) of chemical group 31 (annex I of 1565/2000/EC) structurally related to aliphatic and aromatic hydrocarbons [FEMA 2004-2] used as flavouring substances. 24 June 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.38.

11. EFFA (European Flavour and Fragrance Association), 2005b. Submission 2004-3. Flavouring group evaluation of 32 flavouring substances (candidate chemicals) of chemical group 31 (annex I of 1565/2000/EC) structurally related to aliphatic and aromatic hydrocarbons [FEMA 2004-2] used as flavouring substances. 24 June 2004. FLAVIS/8.38. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to FLAVIS Secretariat.

12. EFFA (European Flavour and Fragrance Association), 2006a. Addendum of 1 flavouring substance (candidate chemical) to the flavouring group evaluation of the chemical group 31 (Annex I of 1565/2000/EC) structurally related to aliphatic and aromatic hydrocarbons [JECFA/WHO FAS 54/63] used as flavouring substances. Addendum to FGE.25 (EFFA submission 2004-3). 21 December 2006. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.106.

13. EFFA (European Flavour and Fragrance Association), 2006b. Addendum of 1 flavouring substance (candidate chemical) to the flavouring group evaluation of the chemical group 31 (Annex I of 1565/2000/EC) structurally related to aliphatic and aromatic hydrocarbons used as flavouring substances. Addendum to FGE.25 (EFFA submission 2004-3). 21 December 2006. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.107.

14. EFFA (European Flavour and Fragrance Association), 2007. E-mail from Jan Demyttenaere, EFFA to FLAVIS Secretariat, National Food Institute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages. FLAVIS/8.70.

15. EFFA (European Flavour and Fragrance Association), 2008. Poundage data on selected substances. Private communication from EFFA to the FLAVIS secretariat. 19 December 2008. FLAVIS/8.113.

16. EFFA (European Flavour Association), 2010. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.

17. EFFA (European Flavour Association), 2011. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark. Dated 11 January 2011. Re.: FGE.25Rev2: FL-no: 01.021 specifications on isomers. FLAVIS/8.118.

18. EFFA (European Flavour Association), 2012. Addendum of Additional Data Relevant to the Flavouring Group Evaluation of the Chemical Group 31 (Annex I of 1565/2000/EC) Aliphatic and Alicyclic and Aromatic Hydrocarbons [JECFA/WHO FAS 54] Structurally Related to Aliphatic and Aromatic Hydrocarbons as Evaluated by EFSA in FGE.25Rev2 and FGE.78Rev1. Addendum to FGE.25Rev2 and FGE.78Rev1. December 2012. FLAVIS/8.176.

19. EFFA (European Flavour Association), 2013. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 6 March and 25 October 2013. Information on substances evaluated in FGE.21Rev4, FGE.25Rev3, FGE.76Rev1, FGE.90Rev1, FGE.93Rev1. FLAVIS/8.185.

20. EFFA (European Flavour Association), 2015. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 13 March 2015. Use levels for substances [FL-no: 01.001, 01.046 and 01.070] in FGE.25Rev3.

21. Flavour Industry, 2006. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-25Rev1.

22. Flavour Industry, 2009. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-25Rev2 [FL-no: 01.001].

23. Flavour Industry, 2010. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-78Rev1/A-25Rev2 [FL-no: 01.008, 01.022, 01.035, 01.047, 01.064].

24. IOFI (International Organization of the Flavor Industry), 1995. European inquiry on volume of use.

25. IOFI (International Organization of the Flavor Industry), 2013. Addendum of Additional Data Relevant to the Flavouring Group Evaluation of the Chemical 31 (Annex I of 1565/2000/EC) Aliphatic and Alicyclic and Aromatic Hydrocarbons [JECFA/WHO FAS 54] Structurally Related to Aliphatic and Aromatic Hydrocarbons as Evaluated by EFSA in FGE.25Rev2 and the Chemical Group 6 (Annex I of 1565/2000/EC) Structurally Related to Aliphatic, Alicyclic and Aromatic Saturated and Unsaturated Tertiary Alcohols, Aromatic Tertiary Alcohols and their Esters [JECFA/WHO FAS 42/51] as Evaluated by EFSA in FGE.18Rev2 Used as Flavouring Substances. Addenda to FGE.25Rev2 and FGE.18Rev2. 30/05/2013. FLAVIS/8.210.

26. Molina-Jasso D, Álvarez-Gonzáles I and Madrigal-Bujaidar E, 2009. Clastogenicity of beta-caryophyllene in mouse. Biological and Pharmaceutical Bulletin 32(3), 520-522.

27. Zook C and Garlick D, 2013. beta-Caryophyllene: a histopathological assessment of the male rat kidney. Histo-Scientific Research Laboratories. Product Safety Labs. Study no. 36206. July 30, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

## REFERENCES

Anderson BE, Zeiger E, Shelby MD, Resnick MA, Gulati DK, Ivett JL and Loveday KS, 1990. Chromosome aberration and sister chromatid exchange test results with 42 chemicals. Environmental and Molecular Mutagenesis 16(Suppl. 18), 55-137.

Asakawa Y, Taira Z, Takemoto T, Ishida T, Kido M and Ichikawa Y, 1981. X-ray crystal structure analysis of 14-hydroxycaryophyllene oxide, a new metabolite of (-)-caryophyllene, in rabbits. Journal of Pharmaceutical Sciences 70(6), 710-711.

Asakawa Y, Ishida T, Toyota M and Takemoto T, 1986. Terpenoid biotransformation in mammals IV. Biotransformation of (+)-longifolene, (-)-caryophyllene, (-)-caryophyllene oxide, (-)-cyclocolorenone, (+)-nootkatone, (-)-elemol, (-)-abietic acid and (+)-dehydroabietic acid in rabbits. Xenobiotica 16(8), 753-767.

Ashley DL and Prah JD, 1997. Time dependence of blood concentrations during and after exposure to a mixture of volatile organic compounds. Archives of Environmental Health 52(1), 26-33.

Austin CA, Shephard EA, Pike SF, Rabin BR and Phillips IR, 1988. The effect of terpenoid compounds on cytochrome P-450 levels in rat liver. Biochemical Pharmacology 37(11), 2223-2229.

Bahima J, Cert A and Menéndez-Callego M, 1984. Identification of volatile metabolites of inhaled n-heptane in rat urine. Toxicology and Applied Pharmacology 76, 473-482.

Bauter MR, 2011. beta-Caryophyllene: palatability/toxicity study: a 14-day dietary study in rats. Product Safety Labs. Study no. 31085. November 16, 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bauter MR, 2013a. beta-Caryophyllene: a 90-day dietary study in rats. Product Safety Labs. Study no. 33328. January 7, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bauter MR, 2013b. Myrcene: a 90-day dietary study in rats. Product Safety Labs. Study no. 33546. May 20, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bogaards JJP, Venekamp JC and van Bladeren PJ, 1996. The biotransformation of isoprene and the two isoprene monoepoxides by human cytochrome P450 enzymes, compared to mouse and rat liver microsomes. Chemico-Biological Interactions 102(3), 169-182.

Brownlee G, 1940. A pharmacological examination of cineole and phellandrene. Quarterly Journal of Pharmacy and Pharmacology 13, 130-137.

Buckley LA, Coleman DP, Burgess JP, Thomas BF, Burka LT and Jeffcoat AR, 1999. Identification of urinary metabolites of isoprene in rats and comparison with mouse urinary metabolites. Drug Metabolism and Disposition 27(7), 848-854.

Chiappe C, De Rubertis A, Amato G and Gervasi PG, 1998. Stereochemistry of the biotransformation of 1-hexene and 2-methyl-1-hexene with rat liver microsomes and purified P450s of rats and humans. Chemical Research in Toxicology 11, 1487-1493.

CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.

Connor TH, Theiss JC, Hanna HA, Monteith DK and Matney TS, 1985. Genotoxicity of organic chemicals frequently found in the air of mobile homes. Toxicology Letters 25, 33-40.

Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. Food and Cosmetics Toxicology 16(3), 255-276.

Crowell PL, Ren Z, Lin S, Vedejs E and Gould MN, 1994. Structure-activity relationships among monoterpene inhibitors of protein isoprenylation and cell proliferation. Biochemical Pharmacology 47(8), 1405-1415.

Dahl AR, 1989. The fate of inhaled octane and the nephrotoxicant, isooctane, in rats. Toxicology and Applied Pharmacology 100, 334-341.

De Meester C, Mercier M and Poncelet F, 1981. Mutagenic activity of butadiene, hexachlorobutadiene, and isoprene. Industrial and Environmental Xenobiotics, Proceedings of an International Conference. 27-30 May 1980, 195-203.

De Rijk EPCT, Ravesloot WTM, Wijnands Y and Van Esch E, 2003. A fast histochemical staining method to identify hyaline droplets in the rat kidney. Toxicologic Pathology 31(4), 462–4.

DeGraff WG, 1983. Mutagenicity evaluation of beta-pinene in the Ames salmonella/microsome plate test. Final report. Litton Bionetics, Inc. LBI project no. 20988. October, 1983. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Del Monte M, Citti L and Gervasi PG, 1985. Isoprene metabolism by liver microsomal monooxygenases. Xenobiotica 15(7), 591-598.

Delgado IF, Carvalho RR, de Almeida Nogueira ACM, Mattos AP, Figueiredo LH, Oliveira SHP, Chahoud I and Paumgartten FJR, 1993a. Study on embryo-foetotoxicity of beta-myrcene in the rat. Food and Chemical Toxicology 31, 31-35.

Delgado IF, de Almeida Nogueira ACM, Souza CAM, Costa AMN, Figueiredo LH, Mattos AP, Chahoud I and Paumgartten FJR, 1993b. Peri- and postnatal developmental toxicity of beta-myrcene in the rat. Food and Chemical Toxicology 31, 623-628.

De-Oliveira A, Ribeiro-Pinto L, Otto S, Goncalves A and Paumgartten F, 1997. Induction of liver monooxygenase by beta-myrcene. Toxicology 124, 135-140.

Di Sotto A, Evandri MG and Mazzanti G, 2008. Antimutagenic and mutagenic activities of some terpenes in the bacterial reverse mutation assay. Mutation Research 653, 130-133.

EC (European Commission), 2000. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2011. Scientific Opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): Aliphatic and aromatic hydrocarbons from chemical group 31. EFSA Journal 2011; 9(6):2177, 126 pp. doi:10.2903/j.efsa.2011.2177

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), in press. Scientific Opinion on Flavouring Group Evaluation 78, Revision 2 (FGE.78Rev2): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2. Not published yet.

EFSA (European Food Safety Authority), 2004a. Minutes of the 7th Plenary Meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. Available online: http://www.efsa.europa.eu/en/events/event/afc040712-m.pdf/.

EFSA, 2004b. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 7: Saturated and unsaturated aliphatic secondary alcohols, ketones, and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids from chemical group 5 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 9 December 2004. EFSA-Q-2003-150.

EFSA (European Food Safety Authority), 2006. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food related to Flavouring Group Evaluation 18 (FGE.18): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical group 6 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). EFSA Journal 2011;9(5):1847, 91 pp.

EFSA (European Food Safety Authority), 2009a. Opinion of the Scientific Panel on Contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Commission related to Flavouring Group Evaluation 43: Thujyl alcohol from chemical group 8 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 26 March 2009. EFSA-Q-2008-047. The EFSA Journal (2009) 1031, 1-38.

EFSA (European Food Safety Authority), 2009b. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food (AFC) related to Flavouring Group Evaluation 78: Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal (2009) 931, 1-59.

Enichem Augusta Ind., 1987. Acute oral toxicity (Acceptance N° 7056 22/9/1987). Cited in European Commission. European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 629-59-4, EINECS Name tetradecane. Section 1.0.1-5.11.

Eriksson K and Levin J-O, 1990. Identification of cis- and trans- verbenol in human urine after occupational exposure to terpenes. International Archives of Occupational and Environmental Health 62, 379-383.

Eriksson K and Levin JO, 1996. Gas chromatographic mass spectrometric identification of metabolites from á-pinene in human urine after occupational exposure to sawing fumes. Journal of Chromatography B 677(1), 85-98.

Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. Available online: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.

Fahrig R, 1984. Genetic mode of action of carcinogens and tumor promoters in yeast and mice. Molecular and General Genetics 194, 7-14.

Falk A, Gullstrand E, Löf A and Wigaeus-Hjelm E, 1990a. Liquid/air partition coefficients of four terpenes. British Journal of Industrial Medicine 47, 62-64.

Falk A, Hagberg M, Lof A, Wigaeus-Hjelm E and Zhiping W, 1990b. Uptake, distribution and elimination of alpha-pinene in man after exposure by inhalation. Scandinavian Journal of Work, Environment and Health 16(5), 372-378.

Falk A, Löf A, Hagberg M, Wigaeus-Hjelm E and Wang Z, 1991. Human exposure to 3-carene by inhalation: toxicokinetics, effects on pulmonary function and occurrence of irritative and CNS symptoms. Toxicology and Applied Pharmacology 110, 198-205.

Falk-Fillipsson A, Löf A, Hagberg M, Wigaeus-Hjelm E and Wang Z, 1993. d-Limonene exposure to humans by inhalation: uptake, distribution, elimination, and effects on the pulmonary function. Journal of Toxicology and Environmental Health 38, 77-88.

Fedtke N and Bolt HM, 1987. The relevance of 4,5-dihydroxy-2-hexanone in the excretion kinetics of nhexane metabolites in rat and man. Archives of Toxicology 61, 131-137.

Felice B, 2005. Acute oral toxicity test acute toxic class method-OECD. Toxikon final report 05-0262-G1. Toxikon corporation Bedford, MA. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Florin I, Rutberg L, Curvall M and Enzell CR, 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 18, 219-232.

Ford RA, Api AM and Letizia CS, 1992. Fragrance raw materials monographs. Longifolene. Food and Chemical Toxicology 30(suppl.), 67S-68S.

Frazier KS, Seely JC, Hard GC, Betton G, Burnett R, Nakatsuji S, Nishikawa A, Durchfeld-Meyer B and Bube A, 2012. Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicologic Pathology 40, 14s-86s.

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin BH, Resnick MA, Anderson B and Zeiger E, 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environmental and Molecular Mutagenesis 10(Suppl. 10), 1-175.

Gocke E, 1999. Mutagenicity evaluation of cedrene washed in the Ames Test. Roche LtD. Project No. 489M99. November 16, 1999. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Gomes-Carneiro MR, Viana MES, Felzenszwalb I and Paumgartten FJR, 2005. Evaluation of beta-myrcene, alpha-terpinene and (+)- and (-)-alpha-pinene in the salmonella/microsome assay. Food and Chemical Toxicology 43, 247-252.

Hard GC, Rodgers IS, Baetchke KP, Richards WL, McGaughy RE and Valcovic LR, 1993. Hazard evaluation of chemicals that cause accumulation of alpha 2u-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environmental Health Perspectives 99, 313-349.

Hart ER and Wong LCK, 1971. Acute oral toxicity studies in rats, acute dermal toxicity and primary skin irritation studies in rabbits of 17 fragrance materials. Bionetics Research Laboratories. July 30, 1971. Report submitted by EFFA to SCF.

Hasegawa M and Toda T, 1978. Teratological studies on Rowachol, remedy for cholelithiasis. Effect of Rowachol administered to pregnant rats during organogenesis on pre-and post-natal development of their offspring. Oyo Yakuri 15(7), 1109-1119. (In Japanese)

Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E, 1983. Salmonella mutagenicity test results for 250 chemicals. Environmental Mutagenesis 5(Suppl. 1), 3-142.

Heck JD, Vollmuth TA, Cifone MA, Jagannath DR, Myhr B and Curren RD, 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9(1), 257-272.

Hiroi T, Miyazaki Y, Kobayashi Y, Imaoka S and Funae Y, 1995. Induction of hepatic P450's in rat by essential wood and leaf oils. Xenobiotica 25(5), 457-467.

Hoechst AG, 1991. Unveroeffentlichte Untersuchung (91.0475). Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 79-92-5, EINECS Name camphene. Section 1.0.1-5.11.

Hoffman-LaRoche Inc., 1967b. Acute toxicity, eye and skin irritation tests on aromatic compounds. Roche Chemical Division. Report no. 30642. September 20, 1967. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Holmberg B, Jakobson I and Sigvardsson KA, 1977. Study on the distribution of methylchloroform and noctane in the mouse during and after inhalation. Scandinavian Journal of Work, Environment and Health 3, 43-52.

Hämäläinen J, 1912. [The conduct of the alicyclical compounds in the glycuronic acid matching in the organism]. Skandinavisches Archiv für Physiologie 27, 141-226. (In German)

IARC (International Agency for Research on Cancer), 1999. Summaries and evaluations, isoprene (group 2b), vol. 71, part three, p. 1015. International Programme on Chemical Safety, International Agency for Research on Cancer, WHO, Lyon.

Igimi H, Kodama M and Ide H, 1974. Studies on the metabolism of d-limonene (p-mentha-1,8-diene). I. The absorption, distribution and excretion of dlimonene in rats. Xenobiotica 4(2), 77-84.

Imbriani M, Ghittori S, Pezzagno G and Capodaglio E, 1985. Urine/air partition coefficients for some industrially important substances. Giornale Italiano di Medicina del Lavoro 7, 133-140.

Ishida T, Asakawa Y, Takemoto I and Aratani T, 1979. Terpene metabolites in rabbit urine. IV. Metabolism of pinan, caran, and myrcene. Koen Yoshishu-Koryo, Terupen 23, 39-41. (In Japanese)

Ishida T, Asakawa Y, Takemoto T and Aratani T, 1981. Terpenoids biotransformation in mammals [I]: biotransformation of alpha-pinene, beta-pinene, pinane, 3-carene, carene, myrcene and p-cymene in rabbits. Journal of Pharmacological Sciences 70(4), 406-415.

Ishida T, Asakawa Y and Takemoto T, 1982. Hydroxyisolongifolaldehyde: A new metabolite of (+)-longifolene in rabbits.Journal of Pharmacological Sciences 71(8), 965-966.

Jagannath DR, 1984. Mutagenicity evaluation of alpha-pinene in the Ames salmonella/microsome plate test. Final report. Litton Bionetics Inc. LBI project no. 20988. January, 1984. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Jauhar PP, Henika PR, MacGregor JT, Wehr CM, Shelby MD, Murphy SA and Margolin BH, 1988. 1,3-Butadiene: induction of micronucleated erythrocytes in the peripheral blood of B6C3F1 mice exposed by inhalation for 13 weeks. Mutation Research 3-4, 171-176.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1993. 41. Report: Forty-First Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Report: WHO Technical Report Series, no. 837.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005a. Compendium of food additive specifications. Addendum 12. Joint FAO/WHO Expert Committee of Food Additives 63rd session. Rome, 8-17 June 2004. FAO Food and Nutrition paper 52 Add. 12.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005b. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8-17 June 2004.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Safety evaluation of certain food additives and contaminants. Sixty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 54. IPCS, WHO, Geneva.

Kanerva RL, Ridder GM, Lefever FR and Alden CL, 1987. Comparison of short-term renal effects due to oral administration of decalin or d-limonene in young adult male Fischer-344 rats. Food and Chemical Toxicology 25(5), 345-353.

Kauderer B, Zamith H, Paumgartten JR and Speit G, 1991. Evaluation of the mutagenicity of beta-myrcene in mammalian cells in vitro. Environmental and Molecular Mutagenesis 18, 28-34.

Khan MA and Heddle JA, 1991. Chemical induction of somatic gene mutations and chromosomal aberrations in lung fibroblasts of rats. Mutation Research 263(4), 257-262.

Kodama R, Noda K and Ide H, 1974. Studies on the metabolism of d-limonene (p-mentha-1,8-diene) - II. The metabolic fate of d-limonene in rabbits. Xenobiotica 4, 85-95.

Kodama R, Yano T, Furukawa K, Noda K and Ide H, 1976. Studies on the metabolism of d-limonene - IV Isolation and characterization of new metabolites and species differences in metabolism. Xenobiotica 6, 377-389.

Kodama R, Okubo A, Araki E, Noda K, Ide H and Ikeda T, 1977a. Studies on d-limonene as a gallstone solubilizer (VII): Effects on development of mouse fetuses and offsprings. Oyo Yakuri 13(6), 863-873. (In Japanese)

Kodama R, Okubo A, Sato K, Araki E, Noda K, Ide H and Ikeda T, 1977b. Studies on d-limonene as a gallstone solubilizer (IX): Effects on development of rabbit fetuses and offsprings. Oyo Yakuri 13(6), 885-898. (In Japanese)

Koppel C, Tenczer J, Tonnesmann U, Schirop T and Ibe K, 1981. Acute poisoning with pine oil-metabolism of monoterpenes. Archives of Toxicology 49, 73-78.

Kurttio P, Kalliokoske P, Lampelo S and Jantunen MJ, 1990. Mutagenic compounds in wood-chip drying fumes. Mutation Research 242, 9-15.

Kushi A, Yoshida D and Mizusaki S, 1985. Mutagenicity of gaseous nitrogen oxides and olefins on Salmonella TA102 and TA104. Mutation Research 147(5), 263-264.

Lankas GR, Baxter CS and Christian RT, 1978. Effect of alkane tumor-promoting agents on chemically induced mutagenesis in cultured V79 Chinese hamster cells. Journal of Toxicology and Environmental Health 4, 37-41.

Lehman-McKeeman LD, Rodriguez PA, Takigiku R, Caudill D and Fey ML, 1989. d-Limonene-induced male rat-specific nephrotoxicity: Evaluation of the association between d-limonene and alpha2u -globulin. Toxicology and Applied Pharmacology 99, 250-259.

Leuschner, 1992. LPT Laboratory of Pharmacology and Toxicology, Report No. 7263/92 (HOE 92.1167). Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 79-92-5, EINECS Name camphene. Section 1.0.1-5.11.

Levenstein I, 1975. Acute oral toxicity (rat - 5gms./kg. body weight dose). Dermal toxicity (rabbit - 5gms./kg. body weight dose). Terpinolene. Leberco Laboratories. Assay no. 51754. January 21, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Levin JO, Eriksson K, Falk A and Lof A, 1992. Renal elimination of verbenols in man following experimental a-pinene inhalation exposure. International Archives of Occupational and Environmental Health 63, 571-573.

Longfellow D, 1998. Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute. Beta-caryophyllene. Mutagenicity studies. Cited in CCRIS.

Longo V, Citti L and Gervasi PG, 1985. Hepatic microsomal metabolism of isoprene in various rodents. Toxicology Letters 29(1), 33-37.

Lorillard (Lorillard Tobacco Company), 1984. Mutagenicity evaluation of beta-caryophyllene in the Ames Salmonella/microsome plate test. Final report. Litton Bionetics, Inc. LBI Project no. 20988. January, 1984. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Low LK, Meeks JR and Mackerer CR, 1987. n-Octane. In: Snyder R (Ed.). Ethel Browning's toxicity and metabolism of industrial solvents. 2nd Ed. Elsevier, Amsterdam, pp. 307-311.

LPT Research, 1992. Report No. 7114/91.Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 79-92-5, EINECS Name camphene. Section 1.0.1-5.11.

Madhusree B, Goto S, Ohkubo T, Tian H, Ando F, Fukuhara M, Tohkin M and Watanabe I, 2002. Mutagenicity testing of 1,3-butadiene, 1,4-pentdiene-3-ol, isoprene, 2,4-hexadiene, cis- and trans-piperlylene. Journal of Health Science 48(1), 73-78.

Madyastha KM and Srivatsan V, 1987. Metabolism of beta-myrcene in vivo and in vitro: its effects on rat-liver microsomal enzymes. Xenobiotica 17, 539-549.

Maltzman TH, Christou M, Gould MN and Jefcoate CR, 1991. Effects of monoterpenoids on in vivo DMBA-DNA adduct formation and on phase I hepatic metabolising enzymes. Carcinogenesis 12(11), 2081-2087.

Manini P, Andreoli R, Bergamaschi E and Franchini I, 1999. Determination of free and glucuronated hexane metabolites without prior hydrolysis by liquid- and gas-chromatography coupled with mass spectrometry. Toxicology Letters 108(2-3), 225-31.

Mennicke WH, Görler K and Krumbiegel G, 1983. Metabolism of some naturally occuring isothiocyanates in the rat. Xenobiotica, 13, 203-297.

Miyazawa M, Shindo M and Shimada T, 2002. Sex differences in the metabolism of (+)- and (-)-limonene enantiomers to carveol and perillyl alcohol derivatives by cytochrome P450 enzymes in rat liver microsomes. Chemical Research in Toxicology 15(1), 15-20.

Molina-Jasso D, Álvarez-Gonzáles I and Madrigal-Bujaidar E, 2009. Clastogenicity of beta-caryophyllene in mouse. Biological and Pharmaceutical Bulletin 32(3), 520-522.

Moreno OM, 1972a. Acute oral toxicity in rats. Myrcene. Toxicological Resources. Project no. 812-72. May 5, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1972b. Acute dermal toxicity in rabbits. alpha-Phellandrene. Toxicological Resources. Project no. 854-72. May 1, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1972c. Acute oral toxicity in rats. 3-Carene. Toxicological Resources. Project no. 843-72. May 5, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1972d. Acute oral toxicity in rats. d-Limonene. Toxicological Resources. Project no. 818-72. May 5, 1970. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1972e. Acute oral toxicity in rats. alpha-Pinene. Toxicological Resources. Project no. 840-72. May 5, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1973a. Acute oral toxicity in rats. Dermal toxicity in rabbits. gamma-Terpinene. MB Research Laboratories, Inc. Project no. MB 73-203. July 23, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1973b. Acute oral toxicity in rats. alpha-Terpinene. MB Research Laboratories, Inc. Project no. MB 73-206. July 25, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1974a. Acute oral toxicity in rats. Dermal toxicity in rabbits. Bisabolene. MB Research Laboratories, Inc. Project no. MB 74-598. August 23, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1974b. Acute oral toxicity in rats. Dermal toxicity in rabbits. Camphene. MB Research Laboratories, Inc. Project no. MB 74-570. June 28, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1976q. Acute dermal toxicity in rats. Dermal toxicity in rabbits. beta-Pinene. MB Research Laboratories, Inc. Project no. MB 75-822. June 25, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1976a. Acute oral toxicity in rats. Dermal toxicity in rabbits. Ocimene. MB Research Laboratories, Inc. Project no. MB 76-1033. March 13, 1976. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1976b. Acute dermal toxicity in rats. Dermal toxicity in rabbits. Guaiene. Project no. MB 76-1222. July 31, 1976. MB Research Laboratories, Inc. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1980. Oral toxicity in rats. Dermal toxicity in rabbits. Valencene. MB Research Laboratories, Inc. Projoct no. MB 80-4726. August 30, 1980. Unpublished data submitted by EFFA to FLAVIS Secretariat.

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 and Molecular Mutagenesis 8(Suppl. 7), 1-119.

Myhr B, McGregor D, Bowers L, Riach C, Brown AG, Edwards I, McBride D, Martin R and Caspary WJ, 1990. L5178Y mouse lymphoma cell mutation assay results with 41 compounds. Environmental and Molecular Mutagenesis 16(Suppl. 18), 138-167.

Müller W, Engelhart G, Herbold B, Jäckh R and Jung R, 1993. Evaluation of mutagenicity testing with Salmonella typhimurium TA102 in three different laboratories. Environmental Health Perspectives (Suppl. 101(3)), 33-36.

Nesslany F, Zennouche N, Simar-Meintieres S, Talahari I, NKili-Mboui E-N and Marzin D, 2007. In vivo Comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds. Mutation Research 630, 28-41.

Nilsen OG, Haugen OA, Zahlsen K, Halgunset J, Helseth A, Aarset H and Eide I, 1988. Toxicity of n-C9 to n-C13 alkanes in the rat on short term inhalation. Pharmacology and Toxicology 62, 259-266.

NTP (National Toxicology Program), 1990. NTP technical report on the toxicology and carcinogenesis studies of d-limonene (CAS no. 5989-27-5) in F344/N rats and B6C3F1 mice (gavage studies). January 1990. NTP-TR 347. NIH Publication no. 90-2802.

NTP (National Toxicology Program), 1999. Toxicology and carcinogenesis studies of isoprene (CAS no. 78-79-5) in F344/N rats and B6C3F1 mice (inhalation studies). NIH Publication no.99-3976. NTP-TR 486. 1-176.

NTP (National Toxicology Program), 2003. Initial study results from a 90-day toxicity study on beta-myrcene in mice. Study number C99023.

NTP (National Toxicology Program), 2010. Toxicology and carcinogenesis studies of beta-myrcene (CAS No. 123-35-3) in F344/N rats and B6C3F1 mice (gavage studies). National Toxicology Program, Research Triangle, NC, USA. TR-557. NIH Publication No. 08-5898. [Online] http://ntp.niehs.nih.gov/.

Olson CT, Yu KO, Hobson DW and Serve P, 1986. The metabolism of n-octane in Fischer 344 rats. Toxicology Letters 31, 147-150.

Opdyke DLJ, 1975. Fragrance raw materials monographs: 1-Hexanol. Alcohol C-6. Camphene. Food and Cosmetics Toxicology 13, 695-696 and 735-738.

Opdyke DLJ, 1978. Fragrance raw materials monographs: beta-Pinene. Food and Cosmetics Toxicology 16(Suppl. 1), 859-861.

Paumgartten FJ, De-Carvalho RR, Souza CC, Madi K and Chahoud I, 1998. Study of the effects of beta-myrcene on rat fertility and general reproductive performance. Brazilian Journal of Medical and Biological Research 31(7), 955-965.

Pellmont B, 1973. Determination af LD50 on mouse and rat. 1,3,5-Undecatriene. June 7, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Perbellini L, Amantini MC, Brugnone F and Frontali N, 1982. Urinary excretion of n-hexane metabolites. A comparative study in rat, rabbit and monkey. Archives of Toxicology 50, 203-215.

Perbellini L, Brugnone F, Cocheo V, De Rosa E and Bartolucci GB, 1986. Identification of the n-heptane metabolites in rat and human urine. Archives of Toxicology 58, 229-234.

Peter H, Wiegand HJ, Bolt HM, Greim H, Walter G, Berg M and Filser JG, 1987. Pharmacokinetics of isoprene in mice and rats. Toxicology Letters 36(1), 9-14.

PETRESA (date not given) Huntingdon Research Centre Report N° 84450D/PEQ/1/AC. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 629-59-4, EINECS Name tetradecane. Section 1.0.1-5.11.

PETRESA (date not given) Huntingdon Research Centre Report N° 84450D/PEQ/1/AC. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 629-59-4, EINECS Name tetradecane. Section 1.0.1-5.11.

Poon G, Chui YC, Law FCP, 1986. Biotransformation of diphenyl ether by trout and guinea-pigs after intraperitoneal administration. Xenobiotica 16(9), 795-800.

Poon GK, Vigushin D, Griggs LJ, Rowlands MG, Coombes RC and Jarman M, 1996. Identification and characterization of limonene metabolites in patients with advanced cancer by liquid chromatography/mass spectrometry. Drug Metabolism and Disposition 24, 565-571.

Rockwell P and Raw I, 1979. A mutagenic screening of various herbs, spices and food additives. Nutrition and Cancer 1(4), 10-15.

Roscheisen C, Zamith H, Paumgartten F and Speit G, 1991. Influence of beta-myrcene on sister chromatid exchanges induced by mutagens in V79 and HTC cells. Mutation Research 264, 43-49.

Sasaki YF, Imanishi H, Ohta T and Yasuhiko S, 1989. Modifying effects of components of plant essence on the induction of sister-chromatid exchanges in cultured Chinese hamster ovary cells. Mutation Research 226, 103-110.

SCF (Scientific Committee for Food), 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

SCF (Scientific Committee for Food), 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I to the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Schreiner CA, 2003. Genetic toxicity of naphthalene: A review. Journal of Toxicology and Environmental Health, Part B 6(2), 161-183.

Sekihashi K, Yamamoto A, Matsumura Y, Ueno S, Watanabe-Akanuma M, Kassie F, Knasmüller S, Tsuda S and Sasaki YF, 2002. Comparative investigation of multiple organs of mice and rats in the comet assay. Mutation Research 517(1-2), 53-75.

Serve MP, Bombick DD, Baughman TM, Jarnot BM, Ketcha M and Mattie DR, 1995. The metabolism of n-nonane in male Fischer 344 rats. Chemosphere 31(2), 2661-2668.

Shapiro R, 1988. Acute toxicity feeding study in rats in a 20% solution of 1,3,5-undecatriene in 80% pinene supra vehicle. Product Safety Labs. Report no. T-8221. August 29, 1988. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Shelby MD, 1990. Results of NTP-sponsored mouse cytogenetic studies on 1,3-butadiene, isoprene, and chloroprene. Environmental Health Perspectives 86, 71-73.

Southwell IAI, Flynn TM and Degrabrielle R, 1980. Metabolism of alpha- and beta-pinene, p-cymene and 1,8-cineole in the brushtail possum. Xenobiotica 10, 17-23.

Tice RR, Boucher R, Luke CA, Paquette DE, Shelby MD and Melnick RL, 1987. Chloroprene and isoprene: Cytogenetic studies in mice with cover letter dated 07/30/87. U.S. EPA. EPA Doc 8EHQ-0887-0689, microfiche no. OTS0513405. Date 8/24/87. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Tice RR, 1988. The cytogenetic evaluation of in vivo genotoxic and cytotoxic activity using rodent somatic cells. Cell Biology and Toxicology 4(4), 475-486.

TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2000. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database. Zeist, The Netherlands. TNO Triskelion, 1963-2000.

TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2010. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database version 12.2/12.3. Zeist, The Netherlands. TNO Triskelion, 1963-2010.

TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2011. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database version 13.1. Zeist, The Netherlands. TNO Triskelion, 1963-2011.

Topping DC, Morgott DA, David RM and O'Donoghue JL, 1994. Ketones. In: Clayton GD and Clayton FE (Eds.). Patty's Industrial Hygiene and Toxicology, 4th Ed. vol. 2C. John Wiley & Sons, Inc., pp. 1739-1878.

Tsuji M, Fujisaki Y, Arikawa Y, Masuda S, Tanaka T, Sato K, Noda K, Ide H and Kikuchi M, 1975a. Studies on d-limonene, as gallstone solubilizer (IV): Chronic toxicity in dogs. Oyo Yakuri 10, 775-808.

Tsuji M, Fujiski Y, Okubo A, Arikawa Y, Noda K, Ide H and Ikeda T, 1975b. Studies on d-limonene as a gallstone solubilizer (V) effects on development of rat fetuses and offsprings. Oyo Yakuri 10,179-186.

Tummey AC, McKee RH and Przygoda RT, 1992. Evaluation of in-vivo tumor promoters in a battery of in-vitro assays. Environmental and Molecular Mutagenesis 19(Suppl. 20), 66.

Turner SD, Tinwell H, Piegorsch W, Schmezer P and Ashby J, 2001. The male rat carcinogens limonene and sodium saccharin are not mutagenic to male Big Blue rats. Mutagenesis 16(4), 329-332.

Vigushin DM, Poon GK, Boddy A, English J, Halbert GW, Pagonis C, Jarman M and Coombes RC, 1998. Phase I and pharmacokinetic study of d-limonene in patients with advanced cancer. Cancer Chemotherapy and Pharmacology 42, 111-117.

Watabe T, Hiratsuka A, Ozawa N and Isobe M, 1981. A comparative study on the metabolism of d-limonene and 4-vinylcyclohex-1-ene by hepatic microsomes. Xenobiotica 11, 333-344.

Webb DR, Ridder M and Alden CL, 1989. Acute and subchronic nephrotoxicity of d-limonene in Fischer 344 rats. Food and Chemical Toxicology 27(10), 639-649.

Webb DR, Kanerva RL, Hysell DK, Alden CL and Lehman-McKeeman LD, 1990. Assessment of the subchronic oral toxicity of d-limonene in dogs. Food and Chemical Toxicology 28, 669-675.

White Jr RA and Agosin M, 1980. Metabolism of alpha-pinene by rat liver reconstituted cytochrome P-450 systems. Development in Biochemistry 13, 85-88.

Zamith HP, Vidal MNP, Speit G and Paumgartten FJR, 1993. Absence of genotoxic activity of beta-myrcene in the in vivo cytogenetic bone marrow assay. Brazilian Journal of Medical and Biological Research 26, 93-98.

Zook C and Garlick D, 2013. beta-Caryophyllene: a histopathological assessment of the male rat kidney. Histo-Scientific Research Laboratories. Product Safety Labs. Study no. 36206. July 30, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

## Appendix A. Procedure for the Safety Evaluation

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), named the "Procedure", is shown in schematic form in Figure A.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996; JECFA, 1997; JECFA, 1999).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 µg/person per day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[16] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[17] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[16] "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

[17] "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

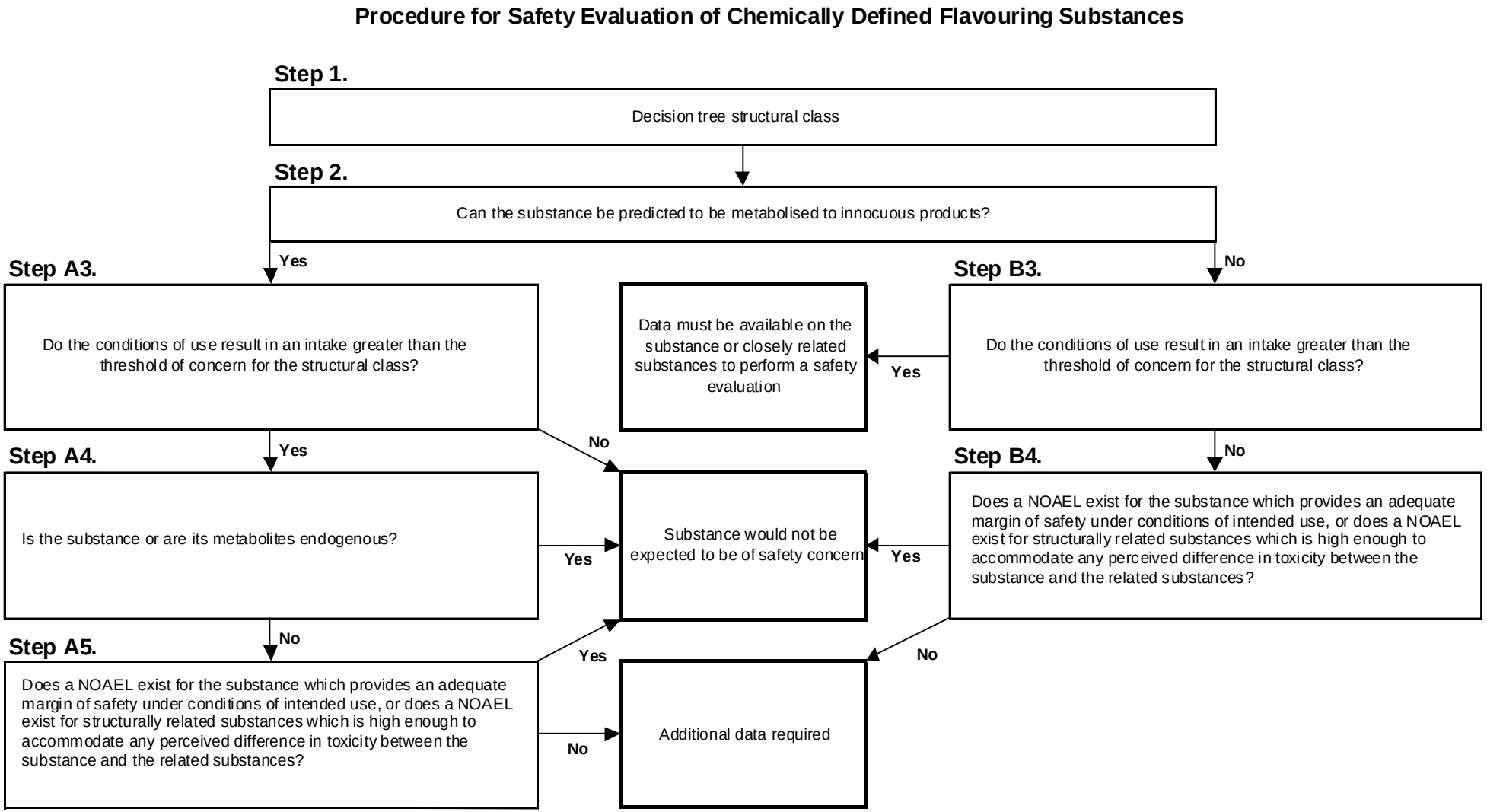



**Figure A.1** *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

## Appendix B. Use Levels / mTAMDI

### B.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table B.1.1) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level” (EC, 2000). According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95$^{th}$ percentile of reported usages (EFFA, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

**Table B.1.1** Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000).

| Food category | Description |
|---|---|
| 01.0 | Dairy products, excluding products of category 02.0 |
| 02.0 | Fats and oils, and fat emulsions (type water-in-oil) |
| 03.0 | Edible ices, including sherbet and sorbet |
| 04.1 | Processed fruit |
| 04.2 | Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds |
| 05.0 | Confectionery |
| 06.0 | Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery |
| 07.0 | Bakery wares |
| 08.0 | Meat and meat products, including poultry and game |
| 09.0 | Fish and fish products, including molluscs, crustaceans and echinoderms |
| 10.0 | Eggs and egg products |
| 11.0 | Sweeteners, including honey |
| 12.0 | Salts, spices, soups, sauces, salads, protein products, etc. |
| 13.0 | Foodstuffs intended for particular nutritional uses |
| 14.1 | Non-alcoholic ("soft") beverages, excl. dairy products |
| 14.2 | Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts |
| 15.0 | Ready-to-eat savouries |
| 16.0 | Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0 |

The “normal and maximum use levels” are provided by Industry for the 12 of the 14 candidate substances in the present flavouring group (Table B.1.2).

**Table B.1.2.** Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.25Rev3 (EFFA, 2005a; EFFA, 2006a; EFFA, 2007).

| FL-no | Food Categories | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Normal use levels (mg/kg)<br>Maximum use levels (mg/kg) | | | | | | | | | | | | | | | | | |
| | **01.0** | **02.0** | **03.0** | **04.1** | **04.2** | **05.0** | **06.0** | **07.0** | **08.0** | **09.0** | **10.0** | **11.0** | **12.0** | **13.0** | **14.1** | **14.2** | **15.0** | **16.0** |
| 01.001 | 5 | 2 | 5 | 1 | 0 | 10 | 5 | 5 | 1 | 1 | 1 | 1 | 2 | 3 | 2 | 5 | 2 | 1 |
| | 25 | 5 | 10 | 1 | 0 | 25 | 10 | 25 | 10 | 5 | 5 | 5 | 20 | 10 | 10 | 10 | 5 | 5 |
| 01.027 | 7 | 5 | 10 | 7 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |
| | 35 | 25 | 50 | 35 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 25 | 50 | 100 | 25 |
| 01.028 | 7 | 5 | 10 | 7 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |
| | 35 | 25 | 50 | 35 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 25 | 50 | 100 | 25 |
| 01.033 | 7 | 5 | 10 | 7 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |
| | 35 | 25 | 50 | 35 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 25 | 50 | 100 | 25 |
| 01.034 | 7 | 5 | 10 | 7 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |
| | 35 | 25 | 50 | 35 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 25 | 50 | 100 | 25 |
| 01.035 | 7 | 5 | 10 | 7 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |
| | 35 | 25 | 50 | 35 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 25 | 50 | 100 | 25 |
| 01.038 | 7 | 5 | 10 | 7 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |
| | 35 | 25 | 50 | 35 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 25 | 50 | 100 | 25 |
| 01.039 | 7 | 5 | 10 | 7 | - | 10 | 4 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |
| | 35 | 25 | 50 | 35 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 25 | 50 | 100 | 25 |
| 01.046 | 3 | 2 | 5 | 1 | 0 | 5 | 2 | 5 | 1 | 1 | 1 | 1 | 2 | 3 | 2 | 4 | 2 | 2 |
| | 15 | 5 | 10 | 1 | 0 | 15 | 10 | 20 | 5 | 5 | 5 | 5 | 15 | 10 | 10 | 10 | 5 | 5 |
| 01.054 | 7 | 5 | 10 | 7 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |
| | 35 | 25 | 50 | 35 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 25 | 50 | 100 | 25 |
| 01.057 | 7 | 5 | 10 | 7 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |
| | 35 | 25 | 50 | 35 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 25 | 50 | 100 | 25 |
| 01.059 | 3 | 2 | 3 | 2 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 3 | 5 | 15 | 5 |
| | 15 | 10 | 15 | 10 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 15 | 8 | 75 | 25 |
| 01.064 | 7 | 5 | 10 | 7 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |
| | 35 | 25 | 50 | 35 | - | 50 | 25 | 50 | 10 | 10 | - | - | 25 | 50 | 25 | 50 | 100 | 25 |
| 01.070 | 3 | 2 | 3 | 2 | 0 | 4 | 2 | 5 | 1 | 1 | 1 | 1 | 2 | 3 | 2 | 4 | 5 | 2 |
| | 15 | 10 | 15 | 10 | 0 | 20 | 10 | 25 | 5 | 5 | 5 | 5 | 10 | 15 | 10 | 20 | 25 | 10 |

## B.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table B.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

**Table B.2.1** Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995).

| Class of product category | Intake estimate (g per day) |
|---|---|
| Beverages (non-alcoholic) | 324.0 |
| Foods | 133.4 |
| Exception a: Candy, confectionery | 27.0 |
| Exception b: Condiments, seasonings | 20.0 |
| Exception c: Alcoholic beverages | 20.0 |
| Exception d: Soups, savouries | 20.0 |
| Exception e: Others, e.g. chewing gum | e.g. 2.0 (chewing gum) |

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000) and reported by the Flavour Industry in the following way (see Table B.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13 and/or 16 (EC, 2000)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

**Table B.2.2** Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995).

| | **Food categories according to Commission Regulation 1565/2000** | **Distribution of the seven SCF food categories** | | |
|---|---|---|---|---|
| Key | Food category | Food | Beverages | Exceptions |
| 01.0 | Dairy products, excluding products of category 02.0 | Food | | |
| 02.0 | Fats and oils, and fat emulsions (type water-in-oil) | Food | | |
| 03.0 | Edible ices, including sherbet and sorbet | Food | | |
| 04.1 | Processed fruit | Food | | |
| 04.2 | Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds | Food | | |
| 05.0 | Confectionery | | | Exception a |
| 06.0 | Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery | Food | | |
| 07.0 | Bakery wares | Food | | |
| 08.0 | Meat and meat products, including poultry and game | Food | | |
| 09.0 | Fish and fish products, including molluscs, crustaceans and echinoderms | Food | | |
| 10.0 | Eggs and egg products | Food | | |
| 11.0 | Sweeteners, including honey | | | Exception a |
| 12.0 | Salts, spices, soups, sauces, salads, protein products, etc. | | | Exception d |
| 13.0 | Foodstuffs intended for particular nutritional uses | Food | | |
| 14.1 | Non-alcoholic ("soft") beverages, excl. dairy products | | Beverages | |
| 14.2 | Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts | | | Exception c |
| 15.0 | Ready-to-eat savouries | | | Exception b |
| 16.0 | Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0 | Food | | |

The mTAMDI values (see Table B.2.3) are presented for each of the 14 flavouring substances in the present flavouring group (EFFA, 2005a; EFFA, 2006a; EFFA, 2007, EFFA, 2015). The mTAMDI values are only given for the highest reported normal use levels.

**Table B.2.3** Estimated intakes based on the mTAMDI approach.

| **FL-no** | **EU Register name** | **mTAMDI (μg/person/day)** | **Structural class** | **Threshold of concern (μg/person/day)** |
|---|---|---|---|---|
| 01.027 | Bisabola-1,8,12-triene | 3900 | Class I | 1800 |
| 01.028 | beta-Bisabolene | 3900 | Class I | 1800 |
| 01.033 | 2,2-Dimethylhexane | 3900 | Class I | 1800 |
| 01.034 | 2,4-Dimethylhexane | 3900 | Class I | 1800 |
| 01.038 | Dodecane | 3900 | Class I | 1800 |
| 01.039 | delta-Elemene | 3900 | Class I | 1800 |
| 01.054 | Pentadecane | 3900 | Class I | 1800 |
| 01.057 | Tetradecane | 3900 | Class I | 1800 |
| 01.035 | 2,6-Dimethylocta-2,4,6-triene | 3900 | Class I | 1800 |
| 01.059 | 4(10)-Thujene | 3100 | Class I | 1800 |
| 01.064 | cis-3,7-Dimethyl-1,3,6-octatriene | 3900 | Class I | 1800 |
| 01.070 | 1-Octene | 1600 | Class I | 1800 |
| 01.001 | Limonene | 1800 | Class I | 1800 |
| 01.046 | l-Limonene | 1600 | Class I | 1800 |

## Appendix C. Metabolism

### C.1. Introduction

This group of flavouring substances is very diverse with respect to the chemical structures. In order to facilitate the evaluation of the metabolism aspects of the individual substances, the candidate substances in the group have been divided into four subgroups: I) acyclic saturated hydrocarbons [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057], II) acyclic unsaturated hydrocarbons [FL-no: 01.001, 01.035, 01.046, 01.064 and 01.070], III) cyclohexene hydrocarbons [FL-no: 01.027, 01.028 and 01.039], V) bicyclic, non-aromatic hydrocarbon [FL-no: 01.059, ]. From the evaluation of flavouring substances as carried out by the JECFA in 2004, a group of supporting substances has been identified. These supporting substances have also been allocated to subgroups in the same way as has been indicated for the candidate substances. The allocation of the candidate and supporting substances is shown in Table C.1.

**Table C.1** Subgroups. The supporting substances are listed in brackets.

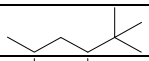
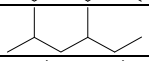
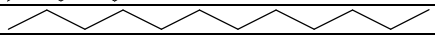
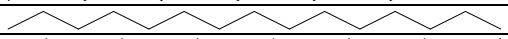
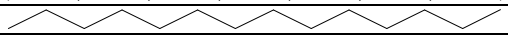
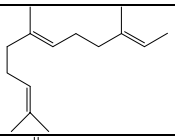
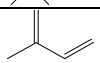
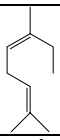
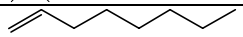
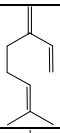
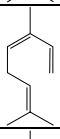
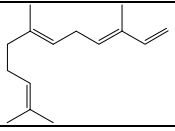
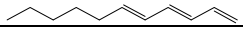
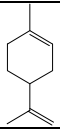
| FL-no | EU Register name | Structural formula | Structural class |
|---|---|---|---|
| **I: ACYCLIC ALKANES** | | | |
| 01.033 | 2,2-Dimethylhexane |  | I |
| 01.034 | 2,4-Dimethylhexane |  | I |
| 01.038 | Dodecane |  | I |
| 01.054 | Pentadecane |  | I |
| 01.057 | Tetradecane |  | I |
| **II: ACYCLIC ALKENES** | | | |
| 01.035 | 2,6-Dimethylocta-2,4,6-triene |  | I |
| Deleted from the Register | 2-Methylbuta-1,3-diene |  | I |
| 01.064 | cis-3,7-Dimethyl-1,3,6-octatriene |  | I |
| 01.070 | 1-Octene |  | I |
| (01.008) | (Myrcene) |  | I |
| (01.018) | (β-Ocimene) |  | I |
| (01.040) | (α-Farnesene) |  | I |
| (01.061) | (Undeca-1,3,5-triene) |  | I |
| **III: CYCLOHEXENE HYDROCARBONS** | | | |
| 01.001 | Limonene |  | I |

**Table C.1** Subgroups. The supporting substances are listed in brackets.

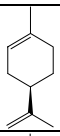
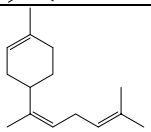
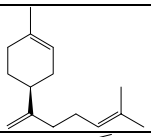
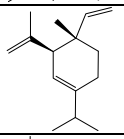
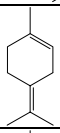
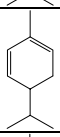
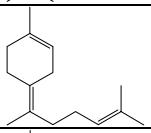
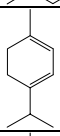
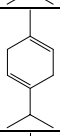
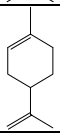
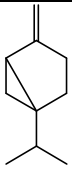
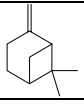
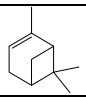
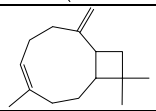
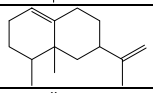
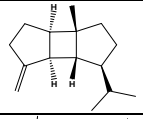
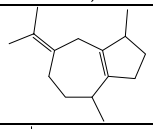
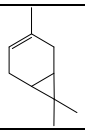
| FL-no | EU Register name | Structural formula | Structural class |
|---|---|---|---|
| 01.046 | l-Limonene |  | I |
| 01.027 | Bisabola-1,8,12-triene |  | I |
| 01.028 | β-Bisabolene |  | I |
| 01.039 | delta-Elemene |  | I |
| (01.005) | (Terpinolene) |  | I |
| (01.006) | (α-Phellandrene) |  | I |
| (01.016) | (1,4(8),12-Bisabolatriene) |  | I |
| (01.019) | (α-Terpinene) |  | I |
| (01.020) | (gamma-Terpinene) |  | I |
| (01.045) | (d-Limonene) |  | I |
| **IV: AROMATIC HYDROCARBONS** | | | |
| The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry | | | |
| **V: BICYCLIC, NON-AROMATIC HYDROCARBONS** | | | |
| 01.059 | 4(10)-Thujene |  | I |
| (01.003) | (Pin-2(10)-ene) |  | I |

**Table C.1** Subgroups. The supporting substances are listed in brackets.

| FL-no | EU Register name | Structural formula | Structural class |
|---|---|---|---|
| (01.004) | (Pin-2(3)-ene) |  | I |
| (01.007) | (β-Caryophyllene) |  | I |
| (01.009) | (Camphene) |  | I |
| (01.017) | (Valencene) |  | I |
| (01.024) | (β-Bourbonene) |  | I |
| (01.026) | (1(5),7(11)-Guaiadiene) |  | I |
| (01.029) | (delta-3-Carene) |  | I |
| **VI: MACROCYCLIC, NON-AROMATIC HYDROCARBONS** | | | |
| The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry | | | |

For the majority of the substances no information of biotransformation had been submitted. Also data on structural analogues were scarce. Therefore, an additional search was carried out. The additional information retrieved has been included in the following text.

## C.2. Absorption, Distribution and Elimination

### Acyclic Alkanes (Candidate and Structurally Related Supporting Substances from Subgroup I)

No data on absorption, distribution and elimination were submitted for any of the candidate substances in this subgroup. Some studies on saturated alkanes were retrieved from the additional data search, but apart from two oral studies (Olson et al., 1986; Serve et al., 1995) and one subcutaneous study (Manini et al., 1999), in all other studies retrieved, inhalation was the route of exposure. From these studies (Bahima et al., 1984; Perbellini et al., 1986; Perbellini et al., 1982; Fedtke and Bolt, 1987; Holmberg et al., 1977), absorption through the membranes of the inhalatory tract was observed. Absorption rates and distribution ratios were dependent on blood/air and blood/tissue partition coefficients (Imbriani et al., 1985; Nilsen et al., 1988). In addition, one inhalation study by (Dahl, 1989) showed that more branched isomers were less well absorbed than less branched or unbranched isomers. More saturated hydrocarbons were less well absorbed than unsaturated and more volatile substances were less well absorbed than less volatile (reversely correlated to chain length), but this may not be a relevant difference for oral exposure situations. The main purpose of these studies was however to study metabolism, but these inhalation exposure studies do in general not provide appropriate mass balance data. For that reason, apart from the two oral and one inhalation study (Dahl, 1989) which give insight in mass balance, the studies by (Bahima et al., 1984; Perbellini et al., 1986; Perbellini et al., 1982; Fedtke and Bolt, 1987) and (Manini et al., 1999) will be discussed in the section on biotransformation.

Male and female rats were given a dose of 2 ml/kg or 1.4 g/kg *n*-octane by gavage and urine was collected for up to 48 hours. Urine samples were treated with glucuronidase/sulphatase and the liberated metabolites were analysed by gas chromatography. Several oxidised metabolites in the urine could be found, but quantitative data were not given (Olson et al., 1986). A similar study was carried out with n-nonane by Serve *et al*. (Serve et al., 1995), but again no quantitative data were provided.

Groups of three or four male F 344 rats were exposed to [4-$^{14}$C] or [5-$^{14}$C]-labelled iso-octane (= 2,2,4-trimethyl pentane (trivial name)) and [1-$^{14}$C]-octane vapours at approximately 1 and 350 ppm (~ 4.76 or 1700 mg/m$^3$ for both substances) by the nose for two hours. During the experiment exhalant was drawn through a bubbler train for sampling. Urine and faeces were collected at the same times, except that none were collected at one and two hours. For iso-octane, all rats exposed to 350 ppm were exposed to C4-labeled substance, but three of four low-exposure rats were exposed to C5-labeled iso-octane. Values for exhaled $^{14}CO_2$ were 0.36; 0.31; and 0.52 % of inhaled $^{14}$C. For the single rat exposed to C4-labeled iso-octane the corresponding value was 2 %[18]. Values for all rats were averaged regardless of position of label. The validity of conclusions regarding low production of $^{14}CO_2$ from iso-octane would have been enhanced by exclusive use of C5-labeled material.

For both *n*-octane and iso-octane the metabolised fraction was higher at low compared to high inhaled concentrations. For octane the major route of elimination was as carbon dioxide (15 % of the radioactive dose within 70 hours). For iso-octane the major route of excretion was urine. Half of the octane-introduced $^{14}$C that was retained at the end of the two-hour exposure period was eliminated 5-10 hours post exposure and the exhalation of radiolabel became undetectable after 30 hours after which 75-85 % of the label was eliminated. For iso-octane the time to eliminate half of the label was 15 hours and was not completed at the end of the observation period. Based on a discussion of papers from other research groups, the study authors suggest that for straight-chain hydrocarbons, breakdown of the carbon skeleton with the release of $CO_2$ is an important metabolic pathway. The route of excretion for *n*-octane in this study was markedly affected by the concentrations of the inhaled vapour. The ratio of total exhaled $^{14}CO_2$: total $^{14}$C in urine was 5:1 after inhalation at 1 ppm but about 1:1 after inhalation of 350 ppm (Dahl, 1989).

**Acyclic Alkenes (Candidate and Structurally Related Supporting Substances from Subgroup II)**

When given to male Japanese White rabbits by gavage at a dose of 670 mg/kg bw per day for two days, approximately 25 % of the total administered amount of myrcene [FL no: 01.008] (19 g to six rabbits) could be recovered from the urine over a period of three days following administration (Ishida et al., 1981). Only metabolites of myrcene were identified. The fate of the remaining part of the dose is unclear.

Following intraperitoneal injection of 64 mg [4-$^{14}$C]-2-methylbuta-1,3-diene /kg to F344 rats and B6C3F$_1$ mice, the majority of radioactivity (≈ 54 and 47 %, respectively) was excreted unchanged in the expired air or as urinary metabolites (≈ 32 and 33 %, respectively) over the 24-hours collection period. Less than 2 % of the radioactivity was recovered as $CO_2$ for both species, and 0.2 and 7.2 % in rats and mice, respectively, of the radioactivity was recovered in the faeces over the same time period. Radioactivity remaining in the carcass and tissues amounted to only 3.1 and 1.7 % in rats and mice, respectively. From the tissues examined, the highest concentration was found in the kidneys but in both species the renal concentration of radioactivity was only twice as high as the concentration in blood. Total percentage of the dose recovered was ≈ 91 % for both rats and mice (Buckley et al., 1999).

[18] In an additional study (not reported in detail), three rats were exposed to C4- or C5-labeled iso-octane at about 350 ppm. More $^{14}CO_2$ was exhaled during exposure to the C4-labeled material (0.07% of the inhaled amount) than after exposure to the C5-labeled material (0.03%). This observation also confirms that C5 labelled iso-octane is less well metabolised to $CO_2$ than C4 labelled iso-octane

**Cyclohexene Derivatives (Candidate and Structurally Related Supporting Substances from Subgroup III)**

Data were only available for one supporting substance in subgroup III, namely *d*-limonene [FL-no: 01.045].

Following the oral administration of [9-$^{14}$C]-*d*-limonene to male Wistar rats by stomach tube at a dose of 800 mg/kg bw, radioactivity was determined in blood, tissues (fat not included), excreta, bile and expired air. The animals were sacrificed at 48 hours post dosing. Radioactivity reached a peak plasma level at two hours post dosing, and after maintaining high levels for 10 hours, declined to negligible levels at 48 hours. In most tissues, peak levels of radioactivity were reached within two hours post dosing indicating rapid distribution. The liver, kidney and adrenals contained the highest levels of radioactivity (higher than blood or serum); other tissues (including brain) contained less than 0.2 % of the administered radioactivity. Hardly any radioactivity could be detected at 48 hours post dosing. Whole body autoradiography confirmed these findings. At 48 hours post-dosing, about 60 % of the administered radioactivity was recovered from the urine, 5 % from faeces and 2 % from exhaled air as $CO_2$. Approximately 25 % of the administered radioactivity was excreted in the bile during 24 hours after administration. Total recovery of radioactivity was less than 100 % and as there was hardly any radioactivity present in the tissues at 48 hours, this could point to loss of volatile $^{14}$C from the excreta or to the elimination of volatile $^{14}$C-compounds other than $CO_2$ (Igimi et al., 1974). When a similar radioactive dose of [9-$^{14}$C]-*d*-limonene was given to male rabbits, 72 % and 7 % of the radioactivity was excreted in the urine and faeces during 72 hours, respectively (Kodama et al., 1974).

In an additional study with several species (rats, hamsters, guinea pigs, rabbits, dogs and humans) dosed orally with [9-$^{14}$C]-*d*-limonene, urinary excretion of radioactivity in rodents and rabbits comprised 82-96 % of the dose within 72 hours and faecal excretion 2-9 %. The total excretion rate in dogs was somewhat lower (77 % *via* urine and 9 % *via* faeces within 72 hours), while two human volunteers excreted 55-83 % of the administered dose in the urine. Faecal excretion in humans was not measured, but may have been considerable in the person with lower urinary excretion as this person developed a diarrhoea shortly after administration. In all species, most excretion occurred within the first 24 hours (Kodama et al., 1976).

*In vitro*, the solubility of *d*-limonene in blood and olive oil was high, but low in water, which suggests a high respiratory uptake and accumulation in adipose tissues (Falk et al., 1990a). Indeed, uptake was rapid and high (68 %) in an experiment in which human volunteers were exposed to *d*-limonene in air at 225 and 450 mg/m$^3$ for 2 hours while doing light physical exercise. The absorbed *d*-limonene was metabolised rapidly. Elimination followed a triphasic pattern, with a short half-life in blood immediately after exposure (2.6 minutes) but a long half-life during the late elimination phase (12.5 hours), which indicates slow elimination from adipose tissues. Approximately 1 % of the total uptake was eliminated unchanged in expired air, while approximately 0.003 % was eliminated unchanged in urine (Falk-Filipson et al., 1993).

**Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup IV)**

The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.

**Bicyclic, Non-Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup V)**

No data on absorption on candidate substances in this group were submitted. Some data were retrieved after database search on longifolene [former FL-no: 01.047] and on several supporting substances including α- and β-pinene [FL-no: 01.004 and 01.003], caryophyllene [FL-no: 01.007] and delta-3-carene [FL-no: 01.029].

Asakawa *et al*. (1986) studied the metabolism of (+)-longifolene and caryophyllene in rabbits after gavage dosing. At two days after administration, metabolites of all of these substances could be detected in the urine, from which it can be concluded that these substances are absorbed. As no mass balance data were given, the extent of absorption cannot be assessed (Asakawa et al., 1986).

Male albino rabbits (6/group) were administered single gavage doses of 400 – 700 mg/kg bw of (+)-α-pinene, (-)-α-pinene, (±)-α-pinene, (-)-β-pinene, or delta-3-carene. Urine of individual animals was collected for three days. The animals excreted bicyclic terpene hydrocarbon metabolites as glucuronic acid conjugates or as further oxidised metabolites, notably carboxylic acids (Schreiner, 2003).

*In vitro* data on the solubility of α-pinene [FL-no: 01.004], β-pinene [FL-no: 01.003] and delta-3-carene [FL-no: 01.029] in blood, olive oil and water suggest a high respiratory uptake and accumulation in adipose tissues. For α-pinene this is supported by a high estimated brain/blood partition coefficient of 18 (Falk et al., 1990a). Experiments in which human volunteers were exposed to (+)- and (-)-α-pinene or delta-3-carene in air at 225 and 450 mg/m$^3$ for two hours while doing light physical exercise confirmed that uptake was rapid and high for these agents (58-60 % for (+)- and (-)-α-pinene and 70 % for delta-3-carene), and that they were metabolized rapidly. Elimination followed a triphasic pattern, with (+)- and (-)-α-pinene exhibiting a rapid initial (distribution) phase (4.8 and 5.6 minutes, respectively), a rapid second distribution phase (38 and 40 minutes, respectively), and a slow elimination phase (695 and 555 minutes, respectively). Triphasic elimination was also observed for delta-3-carene with half-lives of 4.5, 35 and 1800 minutes for the initial, rapid, and slow phases, respectively. It was estimated that it would require over two or six days to eliminate α-pinene or delta-3-carene, respectively, from the body. The long half-lives indicate slow elimination from adipose tissues. Less than 0.001 % of the total uptake of α-pinene or delta-3-carene was eliminated unchanged in the urine, while 7.5-7.8 % and 3 % of the inhaled amount of the α-pinenes and delta-3-carene were exhaled (Falk et al., 1990b; Falk et al., 1991).

In another study, humans were exposed for four or six hours to atmospheres containing a mixture of volatile organic substances, which included α-pinene, at total concentrations of 12 or 24 mg/m$^3$. At a concentration of 24 mg/m$^3$ for the total volatiles, the air concentration of α-pinene was 0.775 mg/m$^3$. The mean pre-exposure blood concentration of α-pinene of 0.035 µg/l increased to an average concentration of 1.9 µg/l during the 4-hours exposure (50-240 minutes). Thereafter (330-450 minutes), the mean blood concentration decreased to 0.15 µg/l. Changes proportional to those observed at 24 mg/m$^3$ were recorded at 12 mg/m$^3$ exposure. Similar results were recorded for the 6-hours exposure. Plasma elimination for α-pinene was best described with a three-exponential curve, with half-lives ranging from 0.22-7.8 minutes, 19-58 minutes and >150 minutes for the initial, mid and terminal phases, respectively (Ashley and Prah, 1997).

In the urine of sawmill workers exposed to an atmosphere containing 31-210 mg/m$^3$ α-pinene [FL-no: 01.004], 2-17 mg/m$^3$ β-pinene [FL-no: 01.003] and 6-90 mg/m$^3$ delta-3-carene [FL-no: 01.029] for three days, *cis* and *trans*-verbenol were identified as metabolites. They were excreted as conjugates, probably with glucuronic acid. The authors suggested that these metabolites were formed by hydroxylation of α-pinene (Eriksson and Levin, 1990). Analysis of urinary metabolites eliminated by human volunteers within four hours following a 2-hours exposure to 10 - 450 mg α-pinene /m$^3$ revealed that α-pinene is indeed eliminated as cis- and trans-verbenol, in a ratio of 1:10, within 20 hours following exposure (Levin et al., 1992). In a more extensive metabolic study, urine was collected from sawmill workers at the end of an eight - nine hours work shift or from chamber-exposed individuals. Following hydrolysis of glucuronic acid conjugates, several pinene biotransformation products were found (Eriksson and Levin, 1996).

**Macrocyclic, Non-Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup VI)**

The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.

## C.3. Conclusions on Absorption, Distribution and Elimination

Based on the available studies it may be concluded that the candidate alkane substances (subgroup I) will be absorbed after oral exposure. The extent of absorption is not known, but may be high, given their lipophilic character and their low molecular weight. The ease with which these substances cross the membranes of the respiratory tract further supports the assumption that these substances will also be absorbed after oral intake. Data indicate that straight-chain alkanes will predominantly be broken down to carbon dioxide at low dose levels. At high dose levels, biotransformation becomes saturated and other metabolites will be generated, which will be excreted via the urine. As illustrated by for iso-octane, metabolism of (highly) branched alkanes to carbon dioxide is less favourable, and their metabolites will be eliminated more slowly via the urine.

For the candidate or supporting substances in subgroup II (acyclic alkenes), data were only available for the supporting substance myrcene [FL-no: 01.008] and for the structurally related substance 2-methylbuta-1,3-diene (former candidate substance (See "History of the evaluation")). Given the narrow range of molecular weights of the candidate substances (all between ~ 68 and 206 D) and their lipophilic nature (Log Kow values e.g. 2.4 (2-methylbuta-1,3-diene), 6.1 (dodec-1-ene), 4.17 (myrcene) or 4.8 (*cis*-3,7-dimethyl-1,3,6-octatriene)), it must be assumed that these candidate flavouring substances will be absorbed from the gastrointestinal tract, at least to some extent, if not completely. Mass balance data are also incomplete. At least for myrcene it has been shown its metabolites will be excreted via urine in an amount of 25 % of the dose within three days after dosing. For 2-methylbuta-1,3-diene a fairly complete mass balance of elimination has been presented, which shows also elimination via the exhaled air (~ 50 % of the dose), but as this substance was administered via the intraperitoneal dose route, the elimination pattern may be different after oral dosing with a lower fraction of the dose exhaled, e.g. because of more efficient first-pass biotransformation after oral dosing.

For the candidate or supporting substances in subgroup group III (cyclohexene derivatives), data were only available for *d*-limonene [FL-no: 01.045], which is a supporting substance. For this substance, data show a considerable absorption from the gastrointestinal tract. In humans, elimination of the substance followed a triphasic pattern, but still 55-83 % of an oral dose could be found in the urine within 72 hours post-dosing, with the major part excreted within 24 hours. Also in other species urinary elimination was most important accounting for up to 82–96 % of the dose within 72 hours. Hence, it may be argued that monocyclic cyclohexene derivatives, such as *d*-limonene, administered orally are absorbed and distributed throughout the body. Following oral administration to humans, *d*-limonene was distributed preferentially to fatty tissues, as indicated by a high oil-blood partition coefficient and a long half-life during the slow elimination phase. Because of the limited molecular weight of the candidate substances in this group (range ~ 136–204 D) and their lipophilic character (e.g. log Kow values for β-phellandrene, β-bisabolene and *d*-limonene are *ca.* 4.7, 7.1 or 4.6, respectively) it may be assumed that all of the substances in this group will be absorbed to some degree, although the extent of absorption for individual substances cannot be accurately estimated from these physico-chemical properties. For the substances in subgroup III with conjugated double bonds no data are available, but based on the structural similarity with the other substances in subgroup III, it may be anticipated that at the substances will also be absorbed.

The substances previously allocated to the subgroup IV are no longer supported for use as flavouring substances in Europe by Industry.

For the substances in subgroup V (bicyclic, non-aromatic hydrocarbons), the available data from oral studies are few. These studies have only addressed the excretion of some supporting and one related substance (longifolene) in the form of metabolites via the urine. No mass-balance data were provided. So these studies only show that these substances will be absorbed to some extent. The same may be anticipated for the (other) candidate substances in this subgroup. For the supporting substances α- and β-pinene and delta-carene information on kinetics is available from humans exposed via inhalation, in occupational settings. These

studies show that these substances can be absorbed after inhalation exposure and that metabolites will be excreted into the urine e.g. as glucuronide conjugates. The elimination follows a triphasic pattern with rather long terminal half-lives. The absorbed amount will be eliminated within several days. Based on the lipophilic character of these substances it may be anticipated that they will preferentially distribute in the adipose tissues, which is supported by the slow terminal elimination rates.

The substances previously allocated to the subgroup VI are no longer supported for use as flavouring substances in Europe by Industry.

## C.4. Metabolism

### Acyclic Alkanes (Candidate and Structurally Related Supporting Substances from Subgroup I)

***Oral***

Male and female rats were given a dose of 1400 mg *n*-octane per kg bw by gavage and urine was collected for up to 48 hours. Urine samples were treated with glucuronidase/sulphatase and the liberated metabolites were analysed by gas chromatography. Compounds found in urine were 2-octanol, 3-octanol, 5-oxohexanoic acid and 6-oxoheptanoic acid. In female rats 2-octanol was found to be the major urinary metabolite and 5-oxohexanoic acid was the major metabolite in males. The authors state that in contrast to *n*-hexane and *n*-heptane, *n*-octane was not metabolised to a ketone, diketone or a diol derivative. The structures of the keto acids that were produced indicate that metabolic oxidation is occurring on both ends of the *n*-octane molecule. A former metabolism study of *n*-heptane did yield *gamma*-valerolactone, which was presumed to have been formed on the GC column from 4-hydroxy-1-pentanoic acid, which would involve a 2 carbon loss. The authors speculate that with *n*-heptane, *n*-octane and perhaps higher straight chain hydrocarbon homologues, a major metabolic pathway involves the formation of acids with loss of carbon (Olson et al., 1986).

The metabolism of n-nonane has been studied in rats after oral administration of 800 mg/kg bw over a 48 hours excretion period. In the urine the following metabolites were detected: 2-, 3- and 4-nonanol, 4-nonanone, 2,5-hexanedione, *gamma*-valerolactone, delta-hexanolactone and delta-heptanolactone and 5-methyl-2-(3-oxobutyl)furane. The authors also reported the formation of 1-heptanol, but no explanation for this metabolite is available. Keto-acids, as observed with n-octane could not be detected after administration on n-nonane (Serve et al., 1995).

***Other Routes of Exposure***

Rats were exposed to n-heptane in a concentration of 8100 mg/m$^3$ in the air for six hours per day five days/week for 12 weeks. After exposure the animals were kept in a metabolism cage to collect urine samples during the next 18 hours for the first week of the experiment and subsequently at the end of every five-day exposure period. Primary metabolites of n-heptane in the urine were 1-, 2-, 3,- and 4-heptanol, of which the 2- and to a lesser extent the 3- isomer were quantitatively the most important. Subsequent oxidations of these two secondary alcohols provided 2- and 3-heptanone and the diols 2,6- and 2,5-heptanediol. In follow-up oxidations, final products were 5-hydroxy-2-heptanone, 6-hydroxy-2-heptanone, 2,6-and 2,5-heptanedione and 6-hydroxy-3-heptanone, which was de-acetylated to yield 4-hydroxy-pentanoic acid. This metabolite was detected in the urine as gamma-valerolactone. The alcoholic metabolites were rapidly excreted as sulphate and glucuronide conjugates. Although 2,5-heptanedione is a gamma-diketone, no signs of peripheral neuropathy were observed. The authors speculated that the plasma levels of this ketone did not become high enough to trigger this effect, because of the rapid conjugation of the precursor alcohols which was supported by the minute amounts in which they were present in the urine, although their (conjugated) precursors reached much higher concentrations (Bahima et al., 1984).

In Sprague-Dawley rats exposed to vapours (7680 mg/m$^3$) of n-heptane for six hours, main urinary metabolites were 2- and 3-heptanol. Other metabolites detected were 2- and 3- heptanone, 4-heptanol, 2,5-heptanedione, gamma-valerolactone, 2,6-dimethyl-2,3-dihydropyrane and 2-ethyl-5-methyl-2,3,-dihydrofurane. In humans, occupationally exposed to n-heptane (5-196 mg/m$^3$) 2- and 3-heptanol were found in the urine. Other metabolites detected were 2- and 4- heptanone and 2,5-heptanedione. In human urinary samples no ring-closure products could be detected, but it is noted that the human exposure was less than that of the rats. In rat tissues (blood, muscle, kidney, nervous tissue, liver), levels of 2-heptanol were between 0.2 and 2 mg/l. Levels of n-heptane in the same tissues were around 20 mg/l (Perbellini et al., 1986).

After oral administration of n-octane (1400 mg/kg bw) to rats in the urine the following metabolites could be detected within 48 hours after dosing: 2- and 3-octanol and 5-keto-hexanoic and 6-keto-heptanoic acid. In contrast to heptane, n-octane was not converted into mono- or diketones or into diols (Olson et al., 1986). Based on this study and the data for n-heptane, the authors argued that for n-heptane and higher straight-chain alkanes, formation of carboxylic acids and subsequent loss of carbon may be a major metabolic pathway. However, the quantitative contribution of de-carboxylation was not thoroughly studied (e.g. no studies with radioactive material) and no mass balances were provided. It has been proposed that n-octane is also converted into 1-octanol and further into octanoic acid, but no detailed information on these studies was submitted (Low et al., 1987).

Groups of three or six rats (Sprague-Dawley, 300-600g), groups of two male rabbits (New Zealand, 1600-2000g) and one male monkey (*Maccaca mulatta*, 1600-2000g) were subjected to single inhalatory treatments of 5000 ppm (~ 18 000 mg/m$^3$) *n*-hexane for 6, 12 and 24 hours. After treatment animals were kept in metabolic cages for the following 72 hours. Urine from treated and control animals were collected during treatment and at various intervals thereafter. For rats blood was collected during and after treatment. Urine samples were subjected to enzymatic and then acid hydrolysis and analysed by gas chromatography. In urine of rats 2-hexanol, 3-hexanol, 2,5-dimethylfurane, *gamma*-valerolactone and 2,5-hexanedione along with methyl *n*-butylketone were found. The metabolites detected in urine from rabbit and monkey were 2-hexanol, 3-hexanol, methyl *n*-butylketone and 2,5-hexanedione. 1-hexanol and 2,5-hexanediol were not detectable in any of the species. In rats peaks of 2,5-hexanedione and 2,5-dimethylfurane in urine were preceded by analogous peaks in blood, while blood concentrations of *n*-hexane, methyl *n*-butylketone and 2-hexanol "peaked" immediately after termination of exposure (Perbellini et al., 1982).

Male rats were exposed to *n*-hexane by inhalation at concentrations ranging from approximately 180 to approximately 11000 mg/m$^3$ for 8 hours in an all-glass exposure system. Urinary kinetics for the metabolites 1-hexanol, 2-hexanol, 3-hexanol, 2 hexanone, 2,5-hexanedione and 4,5-dihydroxy-2-hexanone were

assessed. Urine samples were collected up to 48 hours following the start of exposure. Amounts of metabolites were linearly dependent on the exposure concentrations, up to an exposure of about 1070 mg/m$^3$. Above this the metabolite excretion indicated saturation kinetics in the metabolism. 2-Hexanol amounted to about twice the excretion of all other metabolites excreted. In quantity 4,5-dihydroxy-2-hexanone was the second metabolite, the amount in urine being about 10 times higher than that of excreted 2,5-hexanedione. 2-Hexanol and 4,5-dihydroxy-2-hexanone accounted for 90 % of the total excretion (Fedtke and Bolt, 1987).

A male volunteer was exposed to a mean *n*-hexane atmosphere of 217 ppm (~ 800 mg/m$^3$) for 4 hours. The occurrence of 4,5-dihydroxy-2-hexanone as a metabolite in urine of the man was confirmed. Twenty-six hours after exposure the excretion of 4,5-dihydroxy-2-hexanone reached a level that was four times higher than the excretion of 2,5-hexanedione. The authors conclude that formation of 4,5-dihydroxy-2-hexanone may be viewed as a route of detoxification, and also state that it is clear that 4,5-dihydroxy-2-hexanone is responsible for an artificial appearance of 2,5-hexanedione after drastic acid treatment of human urine, presumably via 2,5-dimethylfuran as intermediate (Fedtke and Bolt, 1987).

Groups of three or four male F 344 rats were exposed to [4-$^{14}$C]-labelled iso-octane (2,2,4-trimethyl pentane) and [1-$^{14}$C]-octane vapours at approximately 1 and 350 ppm (~ 4.76 or 1700 mg/m$^3$ for both substances) for two hours. During the experiment exhalant was drawn through a bubbler train for sampling. Urine and faeces were collected at the same times, except that none were collected at one or two hours. Exhalation of $^{14}CO_2$ appeared to be much more important for n-octane than for iso-octane, from which a larger part of the radioactivity is eliminated via urine. Based on this observation and on a discussion of papers from other research groups, the study authors suggest that for straight-chain hydrocarbons, breakdown of the carbon skeleton with the release of $CO_2$ is an important metabolic pathway. The route of excretion for *n*-octane in this study was markedly affected by the concentrations of the inhaled vapour. The ratio of $^{14}CO_2$: $^{14}$C in urine was 5:1 after inhalation at 1 ppm but 1:1 after inhalation of 350 ppm (Dahl, 1989). The radioactivity eliminated via the urine was not identified.

Hexane was given to rats via subcutaneous injections during three consecutive days. Urinary metabolites were various hexanols, 5-hydroxy-2-hexanone, 4,5-dihydroxy-2-hexanone, 2,5-hexanedione, 2-hexanone and the ring closure products *gamma*-valerolactone and 2,5-dimethylfurane. It was noted that most of the alcoholic metabolites were excreted as conjugates (in particular glucuronide) (Manini et al., 1999). Such conjugation has also been reported in the other alkane biotransformation studies discussed in this Appendix. The study authors (Manini et al., 1999) further demonstrated that the cyclization products (i.e. the lactones and furans) may be formed under the acidic conditions during deconjugation procedures for sample treatment.

*Conclusions on the metabolism of subgroup I substances*

The substances in subgroup I can be expected to be metabolised through omega-oxidation, which will lead to the formation of alcohols, and after oxidation to carboxylic acids, which may be further oxidised via β-oxidation to yield carbon dioxide. Extensive mass balance data are not available, but in some of the studies described above some excretion of carboxylic acids and carbon dioxide has been reported. Formation of keto-acids has also been reported, and these may be formed from omega-oxidation at one end of the molecule and omega-1 or -2 oxidation at the other end. More interest has been put in the identification of metabolites which did not undergo substantial chain shortening. These metabolites will arise from oxidation of the non-terminal carbon atoms, e.g. via omega-1, -2, -3 or even -4 oxidations. The resulting secondary alcohols can be conjugated with e.g. glucuronic acid and excreted via the urine, or can be further oxidised to yield ketones. A subsequent introduction of another secondary hydroxyl group may result in the formation of diketones. With n-hexane, the resulting 2,5-hexanedione (a gamma-diketone) has been demonstrated to be responsible for hexane-induced neurotoxicity. Neurotoxicity is a common feature of gamma-diketones, and for that reason, the candidate substance 3-methylhexane [FL-no: 01.050] cannot be anticipated to be metabolised into innocuous compounds. In addition, it is known that methyl-branching of the carbon chain

potentiates the neurotoxicity of the gamma-diketone (Topping et al., 1994; EFSA, 2004b). The two other hexane derivatives in this group [FL-no: 01.033 and 01.034] cannot be oxidised to gamma-diketone, due to the presence of methyl groups on the C2 and C5 carbon atoms. Therefore, these substances may be metabolised to innocuous metabolites. For the longer chain alkanes in this group, it would require oxidation of the more central carbon atoms in order to be converted into gamma-diketones. Such oxidations are less favourable than omega, omega-1 or -2 oxidations, and therefore it is concluded that oxidation of these higher alkanes will not result in toxicologically relevant levels of gamma-diketones, also because of rapid conjugation of the precursor alcohols. For heptane it was shown that this substance does not result in neurotoxicity (Bahima et al., 1984), although the gamma-diketone itself (2,5-heptanedione) is known to be neurotoxic (Topping et al., 1994). The remaining three candidate flavouring substances in this group i.e. [FL-no: 01.038, 01.054 and 01.057] can be expected to be metabolised to innocuous products.

**Acyclic Alkenes (Candidate and Structurally Related Supporting Substances from Subgroup II)**

For the substances in this group, information is only available on the biotransformation of myrcene ([FL-no: 01.008]; a supporting substance) and 2-methylbuta-1,3-diene (a structurally related substance). Some additional information on the related industrial chemical 1,3-butadiene has also been added. Two of the substances in this group (dodec-1-ene; [FL-no: 01.037] and 1-octene [FL-no: 01.070) bear a terminal double bond without any other structural features (e.g. hydroxyl groups, methyl substituents). No further data for these or similar substances are available. The metabolic aspects of terminal double bond substances have been discussed in FGE.07 and FGE.18 (EFSA, 2004b; EFSA, 2006). The relevant parts of these FGEs will also be included in the current discussion.

*Myrcene [FL-no: 01.008]*

In the urine of rabbits, orally administered myrcene via gavage (670 mg/kg bw per day for two days), 25 % of the total amount administered could be recovered from the urine within three days post-dosing, and > 80 % of the myrcene-derived substances were neutral metabolites; the rest were acidic substances. The main metabolites identified were myrcene-3,10-glycol, myrcene-1,2-glycol and uroterpenol (as acetate) (40.7, 20.8 and 11.8 %, respectively, of the neutral metabolites). Additionally, the glycols underwent further oxidation to yield 2-hydroxymyrcene-1-carboxylic acid and 3-hydroxymyrcene-10-carboxylic acid (no quantitative data were given for these acidic metabolites). The authors suggested that uroterpenol (or limonene-8,9-diol) may have been formed from limonene, which is derived from cyclization of myrcene in the acidic conditions of the rabbit stomach (Ishida et al., 1981). A graphic representation of myrcene metabolites has been presented in Figure C.1.

When rats were administered 800 mg/kg bw per day of myrcene orally via gavage for 20 days, the principal metabolites isolated from the urine were 10-hydroxylinalool (or myrcene-3,10-glycol) and, to a lesser extent, 7-methyl-3-methylene-oct-6-ene-1,2-diol (or myrcene-1,2-glycol). Other minor metabolites included the hydroxy- acids of both the 3,10- and 1,2-glycols (10-carboxylinalool (or 3-hydroxymyrcene-10-carboxylic acid) and 2-hydroxy-7-methyl-3-methylene-oct-6-enoic acid (or 2-hydroxymyrcene-1-carboxylic acid), respectively) and a cyclic diol, 1-hydroxymethyl-4-isopropenylcyclohexanol (or *p*-menth-8-ene-1,7-diol), formed by intramolecular cyclization of an open chain metabolite (Madyastha and Srivatsan, 1987). It was demonstrated that the biotransformation of myrcene was cytochrome P450 (CYP)-mediated and that it could be enhanced by pretreatment of animals with phenobarbital (Madyastha and Srivatsan, 1987).

Aside from being a substrate for CYP enzymes, myrcene has also been shown to induce these enzymes at high dose levels (1000 mg/kg bw per day orally for three days), especially those from the CYP2B (phenobarbital-inducible) subfamily (De-Oliveira et al., 1997). At lower dose levels (40 mg/kg bw per day intraperitoneally for three days) such induction was not observed (Austin et al., 1988).

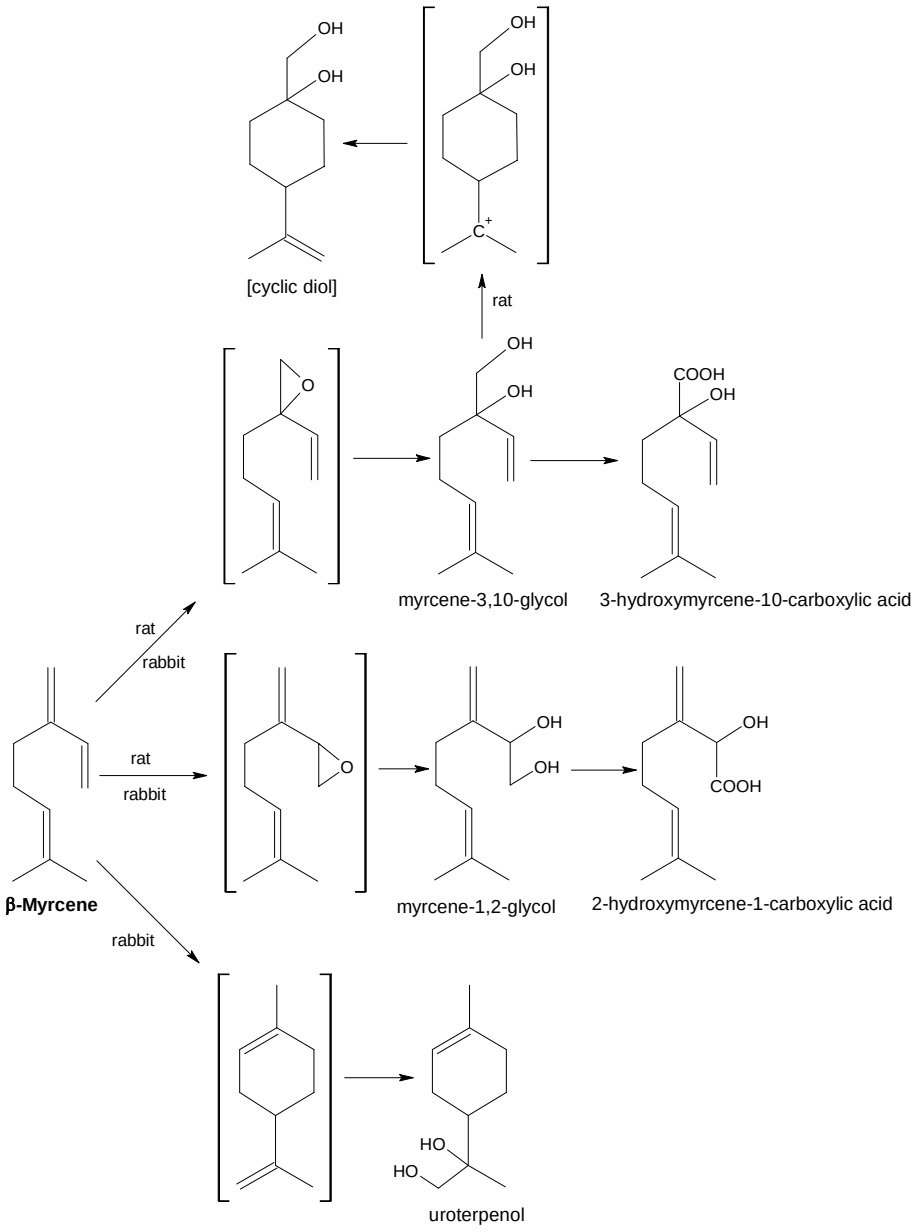



**Figure C.1.** *Metabolism of myrcene in rats and rabbits*

*2-Methylbuta-1,3-diene*

In a sequence of inhalation exposure studies in which rats and mice were exposed to 2-methylbuta-1,3-diene in the air in concentration of 13-11 000 mg/m$^3$, the metabolism of isoprene became saturated at a level of ca. 700 mg/m$^3$ in the rat and ca. 840 mg/m$^3$ in the mouse. Maximal rates of metabolism were 130 micromol/kg bw/hour in the rat and 400 micromol/kg bw/h in the mouse. It was demonstrated that 2-methylbuta-1,3-diene is also produced endogenously at rates of 1.9 micromol/(hour × kg) in rats and 0.4 micromol/(hour × kg) in mice. Part of the endogenous 2-methylbuta-1,3-diene is exhaled by animals, but it is metabolised extensively. The rate of metabolism of endogenously produced and systemically available 2-methylbuta-1,3-diene is 1.6 micromol/(hour × kg) in rats and 0.3 micromol/(hour × kg) in mice, respectively (Peter et al., 1987). The authors quoted literature demonstrating that 2-methylbuta-1,3-diene is also endogenously produced in humans (Gelmont *et al*., 1981 as cited in (Peter et al., 1987)).

Liver microsomes from mice, rats, rabbits and hamsters metabolise 2-methylbuta-1,3-diene to the corresponding monoepoxides, 1,2-epoxy-2-methyl-3-butene (major) and 3,4-epoxy-2-methyl-1-butene (minor). Both monoepoxides are hydrolysed to their respective diols (for chemical structures, see Figure C.2). The main metabolite of 2-methylbuta-1,3-diene (i.e. 1,2-epoxy-2-methyl-3-butene) exhibited a half-life of 75 minutes in aqueous environments; whereas the minor metabolite, 3,4-epoxy-2-methyl-1-butene (14 – 25 % with respect to the main metabolite) is more stable (half-life not specified). The kinetic constants for the formation of the major epoxide metabolite (trans-2-methyl-3-butene-1,2-diol) of 2-methylbuta-1,3-diene were determined in the four test species as apparent Km = 0.06 – 0.2 mM and Vmax = 0.24 – 1.79 nmol trans-2-methyl-3-butene-1,2-diol/mg protein × min. The minor metabolite, 3,4-epoxy-2-methyl-1-butene, was further epoxidised to the diepoxide, 2-methyl-1,2:3,4-diepoxybutane, by microsomes of all rodents studied. The authors argued that the latter diepoxide metabolite could be responsible for the genotoxic and carcinogenic activity of 2-methylbuta-1,3-diene, as the two mono-epoxies were not reported to be genotoxic (Del Monte et al., 1985; Longo et al., 1985).

The *in vitro* metabolism of 2-methylbuta-1,3-diene was investigated in rat, mouse and human liver microsomes and in microsomes derived from cell lines expressing eight different human cytochrome P-450 enzymes. Human CYP2E1 showed the highest rates for formation of the monoepoxides, 1,2-epoxy-2-methyl-3-butene and 3,4-epoxy-2-methyl-1-butene, and CYP2B6 showed the second highest rate. Only CYP2E1 catalysed formation of the diepoxide, 2-methyl-1,2:3,4-diepoxybutane. With human liver microsomes in the presence of an epoxide hydrolase inhibitor, the formation of 1,2-epoxy-2-methyl-3-butene was four times faster than the formation of the 3,4-epoxy-2-methyl-1-butene, which is comparable to the results in rats and mice obtained by others (e.g. Del Monte *et al*., 1985; Longo *et al*., 1985). The rates of monoepoxide formation from isoprene and diepoxide formation from either monoepoxide intermediate were strongly correlated with the microsomal activity of CYP2E1, rather than with the activities of the other CYP enzymes, and both monoepoxides were equally good substrates for the formation of the diepoxide.

Also, species differences with regard to the role of epoxide hydrolase were investigated by comparing the epoxidation of 2-methylbuta-1,3-diene by rat, mouse and human liver microsomes. When an epoxide hydrolase inhibitor was used, similar rates of monoepoxide formation in mouse, rat and human liver microsome systems were measured. However, without epoxide hydrolase inhibition, the total amount of 1,2-epoxy-2-methyl-3-butene measured at the end of the incubation period was twice as high for mouse as for rat liver microsomes and 30 times as high for mouse as for human liver microsomes in which formation of this epoxide was reduced to 4 % of the rate in presence of the inhibitor. For the 3,4-epoxy 2-methyl-1-butene metabolite the effect of epoxide hydrolase inhibition was less dramatic. While hardly any effect was observed in mouse or rat microsomes, in the human microsomes, the rate was reduced to approximately 25 %. The effect of epoxide inhibition on the rate of formation of the diepoxide was not studied. The authors concluded that differences in epoxide hydrolase activity between species may be of crucial importance for the toxicity of 2-methylbuta-1,3-diene in various species (Bogaards et al., 1996).

Following intraperitoneal injection of 64 mg [4-$^{14}$C]-2-methylbuta-1,3-diene per kg to F344 rats, the parent compound was excreted unchanged in the breath (> 50 % of the dose together with < 4 % unidentified material) or via the urine in the form of metabolites (≈ 32 %) over the 24-hours collection period. Only 1.7 % was expired as $CO_2$ and 0.2 % was eliminated via faeces. 3 % remained in the carcass. In the urine 2-methylbuta-1,3-diene was excreted primarily as 2-hydroxy-2-methyl-3-butenoic acid (53 % of total urinary metabolites excreted), 2-methyl-3-buten-1,2-diol (23 %), and the C-1 glucuronide conjugate of 2-methyl-3-buten-1,2-diol (13 %). A fraction of 7 % of the radioactivity in the urine was (an) unidentified polar material. The principal urinary acidic metabolite forms via oxidation of the corresponding 1,2-diol. These metabolites indicate a preferential epoxidation of the methyl-substituted vinyl group of 2-methylbuta-1,3-diene (see Figure C.2) in the rat (Buckley et al., 1999).

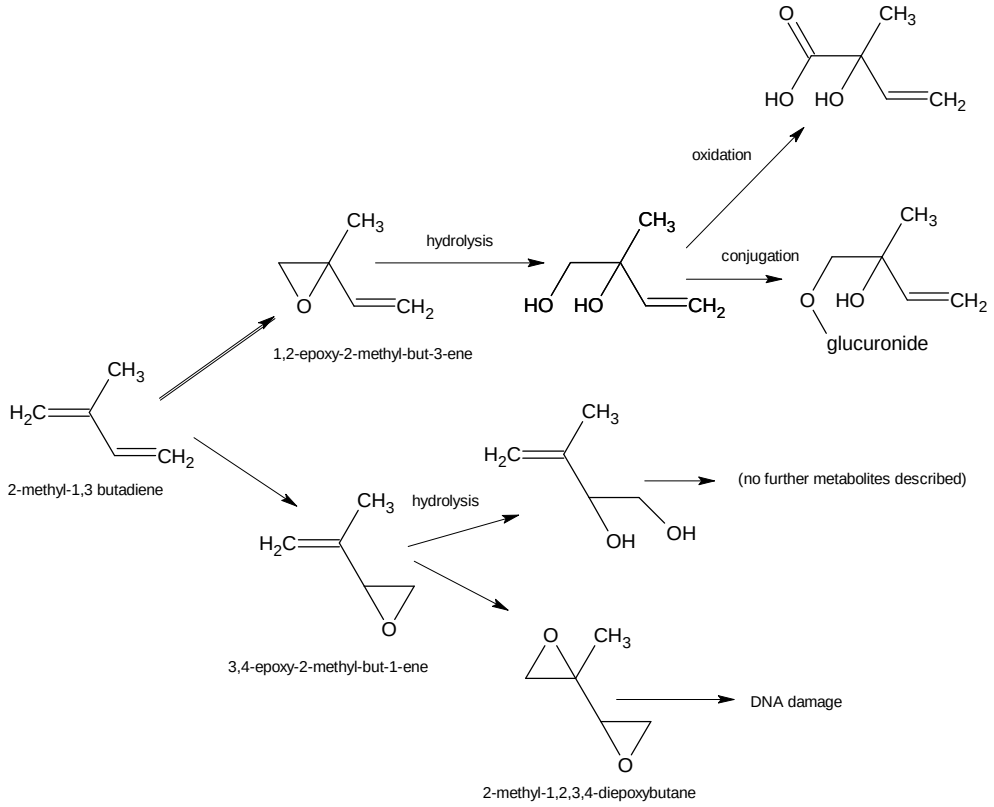



**Figure C.2.** *Major metabolic pathways for 2-methylbuta-1,3-diene (after Del Monte et al. (1985) and Buckley et al. (1999)).*

Following intraperitoneal injection of 64 mg [4-$^{14}$C]-2-methylbuta-1,3-diene /kg to B6C3F$_1$ mice, the parent compound was excreted unchanged in the breath (≈ 44 % of the dose and < 3 % unidentified material) or as urinary metabolites (≈ 33 %) over the 24-hours collection period. Only 1.9 % was expired as $CO_2$ and 7 % was eliminated via faeces. 2 % remained in the carcass. In comparison with rat urine (see above), the urine of the mice contained several other metabolites, including an unidentified polar fraction which comprised 25 % of the total urinary radioactivity as compared to 7 % in rat urine. The major identified metabolite in mouse urine, 2-hydroxy-2-methyl-3-butenoic acid, accounted for ≈ 15 % of the total urinary radioactivity, whereas 2-methyl-3-buten-1,2-diol and its glucuronide conjugate accounted for ≈ 3.5 and 2.5 %, respectively (Buckley et al., 1999). The authors speculated that the unidentified metabolite in the urine of rats and mice could be related to glutathione conjugates. They concluded that if this were the case, in rats glutathione conjugation of 2-methylbuta-1,3-diene is less important than in mice.

*Special features of terminal double bond oxidation*

Double bonds are usually oxidised by P450 to the corresponding epoxides, which are highly reactive molecules. Due to the large strain associated with the three membered ring structure epoxides easily react with nucleophilic sites of cellular macromolecules; conversely they are readily detoxified either spontaneously or by the action of epoxide hydrolase to diols or conjugated with reduced glutathione by glutathione-S-transferases. 1-Alkenes are metabolised by cytochrome P450, through double bond oxidation to the corresponding epoxide or alternatively allylic oxidation (Chiappe et al., 1998). The rates of the two reactions measured with different cytochrome P450 isoforms indicate that epoxide formation is generally favoured (Chiappe et al., 1998) and this may also apply in particular to the candidate substances dodec-1-ene [FL-no: 01.037] and 1-octene [FL-no: 01.070].

Based on this information and the data available for myrcene and 2-methylbuta-1,3-diene, it cannot be excluded that the candidate substances with these terminal double bonds [FL-no: 01.037, 01.064 and 01.070] may be metabolised to epoxides.

*Conclusions on the metabolism of subgroup II substances*

The data available on the metabolism of one supporting and one structurally related substance in subgroup II show that metabolic options for the substances in this group are epoxidation of double bonds ultimately resulting in diols, which can be further conjugated. With the supporting substance myrcene also further metabolism of the diols into carboxylic acids has been reported. Both in rats and rabbits, the principal urinary metabolite following gavage administration of myrcene is myrcene-3,10-glycol, formed from the hydration of the epoxide intermediate in both species. Epoxidation of the 3,10-double bond was favoured over epoxidation of the 1,2-double bond.

The studies indicate that the formation of diols from the myrcene-epoxides is very efficient. It is noted, however, that the diols and the related carboxylic acids are all the result of epoxidation of double bonds in which one of the carbon atoms has only hydrogen substituents, but no further carbon chains. In this respect, the candidate substance cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] is most similar to myrcene. With myrcene no diepoxide metabolites or further reaction products thereof have been reported, but it is noted that mass balance data are incomplete, so some metabolites may have been overlooked.

With the structurally related substance 2-methyl-buta-1,3-diene, epoxidation of the 1,2-double bond is favoured over epoxidation of the 3,4-double bond. While the 1,2-epoxide is readily hydrolysed, the 3,4-epoxide is far more stable. Both metabolites can be converted to the corresponding diols and at least one hydroxy-carboxylic acid (2-hydroxy-2-methyl-3-butenoic acid) has been identified. In addition, in particular the 3,4-epoxide metabolite can be oxidised a second time, resulting in the formation of a diepoxide metabolite, which is known to be reactive and has been suggested to be responsible for 2-methylbuta-1,3-diene-induced DNA damage. For candidate substance dodec-1-ene [FL-no: 01.037], diol- and α-hydroxy-carboxylic acid formation may also be expected. In addition, this substance may also undergo omega and omega-1 or -2 oxidation at the other (saturated) end of the carbon chain (similar to the candidate substances in subgroup I).

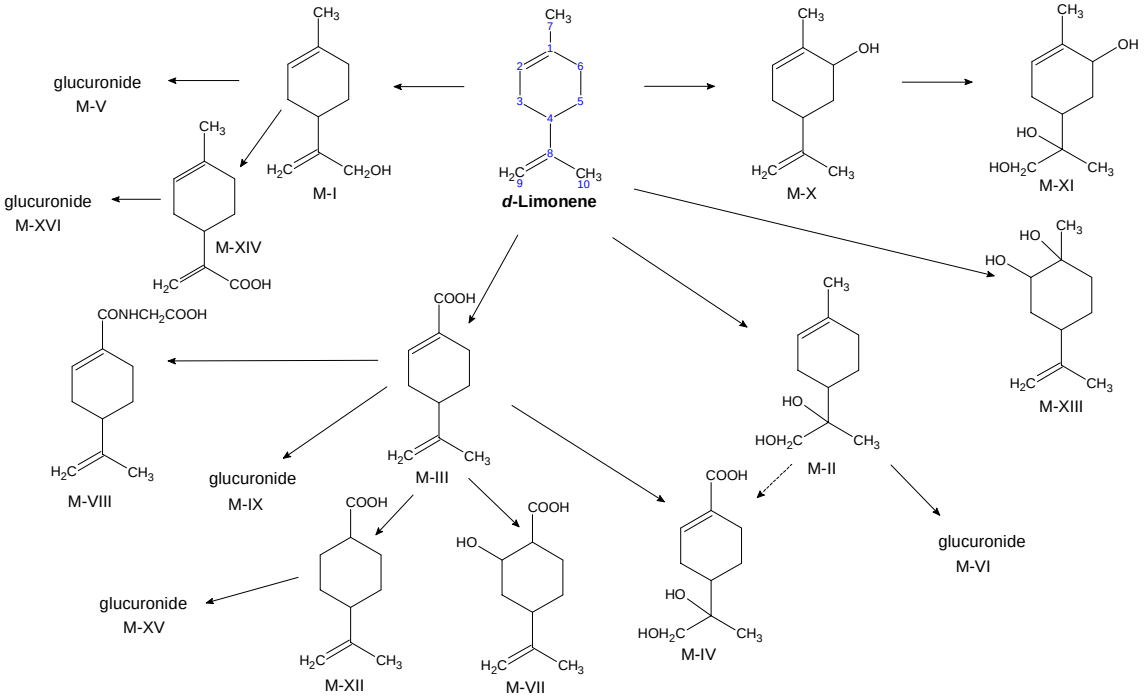
Apart from myrcene and 2-methylbuta-1,3-diene, terminal double bonds appear in candidate substances [FL-no: 01.037, 01.064 and 01.070]. In contrast to corresponding substances in FGE.07 and FGE.18 there is no other functional group in the molecule (e.g. hydroxyl- or keto-group) that could provide a more direct option for detoxication e.g. via conjugation with glucuronide or sulphate. In the two FGEs mentioned above the presence of such groups was an argument to consider that the metabolism of the particular candidate substances would go via innocuous products. It would not be possible to conclude similarly for the candidate substances in FGE.25. The other candidate substances in this subgroup (II) might also be oxidised in the various methyl groups but no data are available to substantiate this. Because of these considerations, it cannot be concluded that the candidate substances in this subgroup [FL-no: 01.032, 01.035, 01.037, 01.064, 01.070 and 01.078] will be metabolised to innocuous products.

**Cyclohexene derivatives (Candidate and structurally related supporting substances from subgroup III)**

For the candidate substances in this group, data for one supporting substance (*d*-limonene; [FL-no: 01.045]) were submitted.

More than 10 metabolites were found in the urine of rats given an oral gavage dose of 800 mg/kg bw *d*-limonene (or *p*-mentha-1,8-diene). Four of the metabolites were identified as perillic acid, *p*-menth-1-ene-8,9-diol (= limonene-8,9-diol), perillic acid-8,9-diol and 8-hydroxy-*p*-menth-1-en-9-yl-glucuronic acid. The bile of these rats contained three metabolites, the most important of which was 8-hydroxy-*p*-menth-1-en-9-

yl-β-D-glucuronic acid (Igimi et al., 1974). Six metabolites were identified in the urine of rabbits given the same oral dose. In addition to the four metabolites identified in rat urine, the rabbit urine contained *p*-mentha-1,8-dien-10-ol (= limonene-10-ol) and *p*-mentha-1,8-dien-10-yl-glucuronic acid (= *p*-mentha-1,8-dien-10-ol-glucuronide). Although not determined quantitatively, perillic acid, perillic acid-8,9-diol and both glucuronic acid conjugates were the major metabolites in rabbit urine, and no unchanged *d*-limonene was detected (Kodama et al., 1974). The same authors identified five additional metabolites in the urine of rats and dogs treated orally with *d*-limonene. These were characterised as 2-hydroxy-*p*-menth-8-en-7-oic acid, perillylglycine, perillyl-glucuronide, *p*-mentha-1,8-dien-6-ol (or limonene-6-ol) and *p*-menth-1-ene-6,8,9-triol. They also found some species differences in the nature of the major metabolites in urine. Perillic acid-8,9-diol was the main metabolite in rats and rabbits, perillyl-glucuronide in hamsters, limonene-8,9-diol in dogs and limonene-8,9-diol-glucuronide in guinea pigs and humans. It should be noted that the fate of only 40-65 % of the *d*-limonene dose administered orally to these animals and humans was accounted for (Kodama et al., 1976). The metabolites of *d*-limonene are shown in Figure C.3.




| | | | |
|---|---|---|---|
| M-I | limonene-10-ol | M-IX | glucuronide conjugate of M-III |
| M-II | limonene-8,9-diol | M-X | limonene-6-ol |
| M-III | perillic acid | M-XI | *p*-menth-1-ene-6,8,9-triol |
| M-IV | perillic acid-8,9-diol | M-XII | dihydroperillic acid |
| M-V | glucuronide conjugate of M-I | M-XIII | limonene-1,2-diol |
| M-VI | glucuronide conjugate of M-II | M-XIV | *p*-mentha-1,8-diene-10-carboxylic acid |
| M-VII | 2-hydroxy-*p*-menth-8-en-7-oic acid | M-XV | glucuronide conjugate of M-XII |
| M-VIII | perillylglycine | M-XVI | glucuronide conjugate of M-XIV |

**Figure C.3.** *Metabolism of d-limonene*

Perillic acid, dihydroperillic acid and limonene-1,2-diol were the major metabolites identified in the plasma of humans given an oral dose of *d*-limonene. Minor metabolites were the methyl esters of perillic acid and dihydroperillic acid, and *d*-limonene itself (Crowell et al., 1994). Apart from the parent compound (Poon et al., 1996) and (Vigushin et al., 1998) also identified perillic acid, dihydroperillic acid and limonene-1,2-diol

as major metabolites in human plasma. However, they also found two other metabolites, i.e. *p*-mentha-1,8-diene-10-carboxylic acid and limonene-8,9-diol, whereas they did not detect the methyl esters of perillic acid and dihydroperillic acid. Peak plasma levels for all metabolites were achieved four to six hours after administration, with the exception of limonene-8,9-diol which reached its peak level one hour after administration (Poon et al., 1986). Metabolites in human urine comprised the glucuronic acid conjugates of perillic acid, dihydroperillic acid, *p*-mentha-1,8-diene-10-carboxylic acid, limonene-8,9-diol and limonene-10-ol (Poon et al., 1986).

Experiments with rat liver microsomes have shown that epoxidation of the C8 double bond (in the vinyl substituent) of *d*-limonene is favoured over epoxidation of the C1-double bond (the one in the ring), due to steric hindrance by the 1-methyl group, which was demonstrated by comparison of the metabolism of *d*-limonene with that of 4-vinylcyclohex-1-ene. Upon incubation with rat liver microsomes, the majority of *d*-limonene was converted to the 8,9-epoxide and the 8,9-diol, and to a much lesser extent to the 1,2-epoxide and the 1,2-diol (ratio of 8,9- vs.1,2-epoxidation = 4:1). In contrast, with 4-vinylcyclohex-1-ene the epoxidation rate of the ring double bond was about four times as fast as the epoxidation rate of the vinyl double bond. Because the 1,2-epoxide of *d*-limonene is a very poor substrate for microsomal epoxide hydrolase, the 1,2-diol could not be found in microsomal incubates, whereas the 8,9-diol could be found (Watabe et al., 1981). Both epoxides of *d*-limonene were tested for mutagenicity in several *Salmonella* strains and showed to be inactive. The mono-epoxides of 4-vinylcyclohex-1-ene were inactive in *Salmonella* strain TA100, but were not tested in the other strains. However, the diepoxide of 4-vinylcyclohex-1ene was mutagenic in this bacterial strain, but this diepoxide was not detected in the microsomal metabolism studies with 4-vinylcyclohex-1-ene as starting material (Watabe et al., 1981).

Other *in vitro* experiments have shown that male rats can convert *d*- and *l*-limonene into the corresponding *trans*-perillyl alcohol (by hydroxylation of the methyl group at C7) and carveol (or limonene-6-ol; by ring C6-hydroxylation). These reactions are catalysed by CYP2C11 and, when pretreated with phenobarbital, CYP2B1. In female rats, the activity for conversion to either alcohol is much lower. Apparently, the female-specific CYP2C12 has no activity with respect to *d*- and *l*-limonene hydroxylation. In males, the hydroxylation activities were not detectable with foetal liver microsomes, but they increased after birth, closely related to the developmental increase in CYP2C11. This study also investigated whether the *d*- and *l*-limonene enantiomers are differently metabolised by liver microsomes. Both in liver microsomes from untreated and treated with phenobarbital, approximately the same amounts of carveol and perillyl alcohol were formed from the two limonene enantiomars. Ratios over the two routes of metabolism were carveol/perillyl alcohol: 0.87/1.23 for *d*-limonene and 0.61/1.03 for *l-limonene*. Also the rate of formation of carveol and perillyl alcohol from *d*- and *l*-limonene is similar using either liver microsomes or recombinant P450 enzymes (Miyazawa et al., 2002).

In male rats orally administered 3 mmol/kg (408 mg/kg) of [$^{14}$C]-*d*-limonene radioactivity was detected in the renal cytosol. Forty percent of the total cytosolic radioactivity was reversibly associated with the protein fraction and further analysis showed that > 97 % of this activity was associated with one single protein, which was identified as α2u-globulin. 1,2-Limonene epoxide, 1,2-limonene-diol and *d*-limonene comprised 82, 5 or 13 %, respectively, of the radioactivity associated with this protein (Lehman-McKeeman et al., 1989).

*d*-Limonene has been shown to induce P450 enzymes of the CYP2B and CYP2C subfamilies and epoxide hydrolase in rats (Austin et al., 1988; Maltzman et al., 1991).

*Conclusions on the metabolism of subgroup III substances*

In the subgroup III there are only metabolism data available for one supporting substance, *d*-limonene, which in several animal species and humans has been demonstrated to be oxidised in both side chains and at the cyclohexene ring, resulting in alcohols and/or carboxylic acids. Ring and side chain hydroxylation has also

been described for its structural isomer *l*-limonene (a constituent of candidate substance [FL no: 01.001] and a candidate substance on its own [FL-no: 01.046]) in rat liver microsomes. The metabolites of these limonenes are, at least partly, conjugated and eliminated with the urine. It is anticipated that three out of the six candidate substances [FL-no: 01.027, 01.028 and 01.039] in subgroup III are metabolised in a similar way to innocuous products. It cannot be anticipated based on the data available that β-phellandrene [FL-no: 01.055] with a double bond directly on the ring and in conjugation with double bond in the cyclohexene ring can be metabolised to innocuous products.

**Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup IV)**

The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.

**Bicyclic Non-Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup V)**

Analysis of urinary metabolites eliminated by human volunteers within four hours following a 2-hour inhalation exposure to 10 – 450 mg (+)-α-pinene/$m^3$ [FL-no: 01.004] in a pharmacokinetic study (Falk *et al*., 1990b) revealed *cis*- and *trans*-verbenol in a ratio of 1:10, with 3.8 and 1.7 % being eliminated at 10 and 450 mg/$m^3$, respectively. Most of the verbenols were eliminated within 20 hours. In a more extensive metabolic study, urine was collected from sawmill workers at the end of an 8-9 hours work shift or from chamber-exposed individuals. Following hydrolysis of glucuronic acid conjugates, *cis*- and *trans*-verbenol were identified in the urine along with two diols, *cis*- and *trans*-4-hydroxymyrtenol, formed by methyl group hydroxylation of *cis*- and *trans*-verbenol. *trans*-4-Hydroxymyrtenal was also detected (see Figure C.4) (Eriksson and Levin, 1990).

Analysis of the urinary metabolites of a patient attempting suicide with 400 – 500 ml pine oil containing 57 % α-pinene showed the presence of myrtenol, verbenol, and borneol. Renal excretion reached a peak level five days after ingestion (Koppel et al., 1981).

Male albino rabbits (six/group) administered single gavage doses of 400 – 700 mg/kg bw of (+)-α-pinene, (-)-α-pinene, (+/-)-α-pinene, (-)-β-pinene, or delta-3-carene, excreted bicyclic terpene hydrocarbon metabolites as (glucuronic acid) conjugates or as further oxidised metabolites, notably carboxylic acids. Animals were housed individually and urine was collected daily for three days.

The principal neutral metabolite formed by oxidation at the $C_4$ position in the alicyclic ring of each of the three α stereochemical forms of pinene was *trans*-verbenol (see Figure C.4). As a minor pathway, allylic oxidation of the exocyclic methyl group to yield myrtenol was observed for all three α-pinene stereoisomers, with also myrtenic acid as minor metabolite (Ishida et al., 1981).

The presence of an exocyclic alkene function in (-)-β-pinene provided additional metabolic options, and four neutral and one acidic metabolites were identified. Allylic oxidation of the methyl group at the $C_2$ position yields (+)-trans-pinocarveol, while epoxidation of the exocyclic alkene followed by hydration or rearrangement yields (-)-*trans*-10-pinanol and (-)-1-*p*-menthene-7,8-diol, respectively. Ring cleavage yields (-)-α-terpineol. These metabolites comprised 11, 39, 30 or 5 % of the total urinary neutral metabolite fraction, respectively. The acidic metabolite identified was identical to the one identified for the α-pinenes (i.e. myrtenic acid), which was suggested to be formed via double bond epoxidation and subsequent rearrangement to give myrtenol and further oxidation to the carboxylic acid (Ishida et al., 1981).

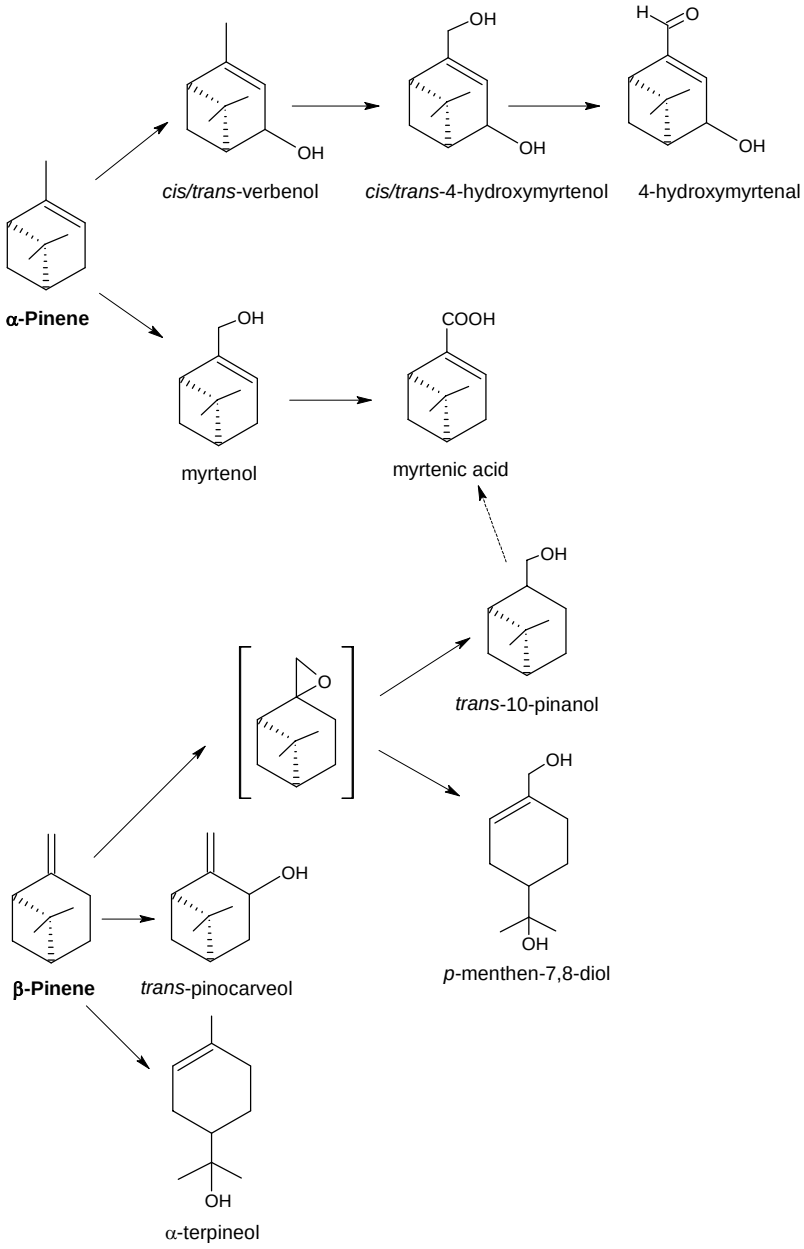



**Figure C.4.** *Metabolism of α-pinene and β-pinene in animals*

*Trans*-verbenol and myrtenic acid have also been found in faeces or urine of brushtail possum fed α-pinene. When the same species was fed β-pinene, only myrtenic acid was found in the excreta (Southwell et al., 1980).

It has been stated in a limited review paper that in rabbits, α- and β-pinenes can be excreted as glucuronide conjugates of undetermined nature, which can release cymene upon heating in diluted acids (Williams, 1959 as cited in (Opdyke, 1978)).

Delta-3-Carene [FL-no: 01.029] undergoes stereoselective hydroxylation at the *gem*-methyl group (yielding 3-caren-9-ol) followed by carboxylation, allylic oxidation of the $C_{10}$ methyl group followed by carboxylation or, as the main route, allylic ring opening and hydroxylation at a secondary carbon atom, yielding (-)-*m*-

mentha-4,6-dien-8-ol (72 % of the total urinary neutral metabolite fraction) and *m*-cymen-8-ol (Ishida et al., 1981).

In addition to the terpenoids pinene and carene, the group of Ishida has also studied the metabolism of the saturated analogues pinane and carane[19] (Ishida *et al*., 1981). Only a relatively small part of the dose (in total 18 g given to six rabbits) was identified, among which were 3- and 4-pinalol, α-terpineol, trans-sobrerol, trans-carveol, and verbenol (some of the structures have been shown in Figure C.4). In short, these products result from hydroxylations of secondary carbon atoms in the 6-membered ring, or cleavage of the 4-membered ring in combination with hydroxylation in the remaining 6-membered ring. In addition, desaturation of the 6-membered ring was also observed and hence, some of the metabolites may be considered as cyclohexene derivatives. No hydroxylation of the primary carbon atom (i.e. the methyl ring substituent) in pinane was observed. With carane, however, the hydroxylation of one of the *gem*-methyl groups and hydroxylation of the C10 exocyclic carbon atom was observed, ultimately resulting in the formation of carane-9,10-dicarboxylic acid.

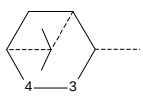
In rabbits, β-caryophyllene [FL-no: 01.007] undergoes epoxidation of the endocylic 5,6-double bond to yield a stable epoxide metabolite and hydroxylation at the *gem*-dimethyl group. The resulting metabolite 14-hydroxycaryophyllene-5,6-epoxide and its C14-acetylated conjugate could be detected in the urine. A second epoxidation of the 5,6-epoxide's exocyclic 2,12-double bond, ultimately resulting in the 14-hydroxycaryophyllene-5,6-epoxide-2,12-diol, was also reported (Asakawa et al., 1981; Asakawa et al., 1986).

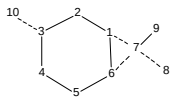
Data were also found on the metabolism of camphene[20]. Ishida *et al*. (1979) administered *dl*-camphene [FL-no: 01.009] to five starved male rabbits at a dose level of 800 mg/kg bw via stomach tube. In total 8 g of the substance was given. Urinary metabolites, collected over three days post-dosing were examined. The following metabolites were identified in urine samples after treatment with β-glucuronidase/sulphatase: camphene-2,10-glycol (after epoxidation of the double bond), 6-*exo*-hydroxycamphene, 7-hydroxycamphene, 10-hydroxytricyclene and 3-hydroxytricyclene. Quantitative information was provided only for the diol which was found in a total amount of 260 mg which corresponds to *ca.* 3 % of the dose (Ishida et al., 1979).

In a human volunteer and in a young pig, camphene was eliminated from the body by exhalation and via bile as unchanged substance or as glucuronide conjugate via the urine (Opdyke, 1975).

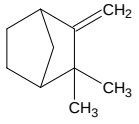
(+)-Longifolene [former FL-no: 01.047] metabolism was studied following the oral administration of about 2 g/animal as a suspension in 0.02 % Tween80 aqueous solution to six male rabbits. Urine samples were collected daily for three days and treated with β-glucuronidase / sulphatase. Due to imprecise description of dose and amounts recovered, it is not possible to indicate the extent of metabolism and excretion as percentage of the dose. Deconjugated metabolites were extracted in a neutral and an acidic fraction. In the neutral fraction (10 % of the dose), many peaks were observed but only one was further characterised to be (2S, 7S)-(+)-14-hydroxyisolongifolaldehyde (35 % of the neutral metabolite fraction). It was concluded that

19

Pinane: 


Carane: 


20 camphene: 3,3-dimethyl-2-methylenenenorbornane



(+)-longifolene is metabolised at two sites in two subsequent steps: 1) oxidation of the *exo*-methylene group to form its epoxide with subsequent isomerisation to form a stable aldehyde, and 2) hydroxylation of the *gem*-dimethyl group to form a primary alcohol (Ishida et al., 1982; Asakawa et al., 1986; Ford et al., 1992).

An *in vitro* study with rat liver microsomes demonstrated the involvement of cytochrome P450 enzymes in the metabolism of α-pinene. Metabolites present were β-pinene and *d*-limonene together with smaller amounts of *trans*-verbenol, myrtenol, verbenone, and pinene oxide (White and Agrosin, 1980).

α-Pinene and cadinene, have been shown to induce cytochrome P450 enzymes, especially those from the CYP2B subfamily, and to a lesser extent also CYP3A2 (cadinene) and CYP4A2 (α-pinene) (Austin et al., 1988; Hiroi et al., 1995). Based on similarity with β-pinene it may be speculated that the candidate substance 4(10)-thujene [FL-no: 01.059] may be hydroxylated to thujyl alcohol, which is known to be conjugated with glucuronic acid and eliminated via urine (Hämäläinen, 1912; EFSA, 2009a). However, based on the same similarity, epoxidation of the exocyclic double bond may also be expected, and it is not clear what other reactions might occur. No indications of the relevance of the various routes are available.

*Conclusions on the metabolism of the subgroup V substance*

Metabolism data for subgroup V are available for some supporting substances (pinenes, camphene, caryophyllene, delta-3-carene, pinane and carane) and longifolene [former FL-no: 01.047]. In general the metabolic options for these substances include oxidation of methyl ring substituent groups to give the corresponding alcohol and further oxidation products. For the substances studied, double bond epoxidation has also been demonstrated. In addition, ring cleavage has also been observed, e.g. for β-pinene resulting in the formation of monocyclic terpenoid derivatives like α-terpineol. Hydroxylated metabolites (i.e. alcohols) or further metabolic products may be eliminated as conjugates e.g. with glucuronic acid. However, given the diversity of this group, the lack of data on any of the candidate substances, it cannot be concluded that the candidate substance in this subgroup [FL-no: 01.059] can be metabolised to innocuous products.

**Macrocyclic, Non-Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup VI)**

The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.

## C.5. Summary on Absorption, Distribution, Metabolism and Excretion

Given the diverse nature of the chemical structures of the candidate flavouring substances in this FGE, it is impossible to draw conclusions, which are applicable to all substances. The amount on information on kinetics of either candidate or supporting substances is very limited. For these reasons, the available information is presented here in a subgroup-wise manner.

**Absorption, Distribution and Elimination**

*Subgroup I*

From the available studies it may be concluded that the candidate alkane substances in subgroup 1 will be absorbed after oral exposure. The extent of absorption is not known, but may be high, given their lipophilic character and their low molecular weight. The ease with which these substances cross the membranes of the respiratory tract further supports the assumption that these substances will also be absorbed after oral intake. Data indicate that straight-chain alkanes will predominantly be broken down to carbon dioxide at low dose levels. At high dose levels, biotransformation becomes saturated and other metabolites will be generated which will be excreted via the urine.

*Subgroup II*

For the candidate and supporting substances in subgroup II, the acyclic alkenes, data were only available for myrcene [FL-no: 01.008] and 2-methylbuta-1,3-diene (a structurally related substance). Given the narrow range of molecular weights of the candidate substances (between ~ 68 and 206 D) and their lipophilic nature (estimated or measured Log Kow values e.g. 2.4 (2-methylbuta-1,3-diene), 6.1 (dodec-1-ene), 4.17 (myrcene) or 4.8 (cis-3,7-dimethyl-1,3,6-octatriene)), it is assumed that these candidate flavouring substances may be absorbed from the gastrointestinal tract. Mass balance data for myrcene are incomplete. For 2-methylbuta-1,3-diene a mass balance of elimination has been presented, which also shows elimination via the exhaled air (~ 50 % of the dose), but as this substance was administered via the intraperitoneal route, the elimination pattern may be different after oral dosing with a lower fraction of the dose exhaled, e.g. because of more efficient first-pass biotransformation after oral dosing.

*Subgroup III*

For the candidate and supporting substances in subgroup III (cyclohexene derivatives), data were only available for *d*-limonene [FL-no: 01.045]. For this substance, data show a considerable absorption from the gastrointestinal tract. In humans, elimination of the substance followed a triphasic pattern, but still 55-83 % of an oral dose could be found in the urine within 72 hours post dosing, with the major part excreted within 24 hours. Also in other species urinary elimination was most important accounting for up to 82-96 % of the dose within 72 hours. Hence, it may be anticipated that monocyclic cyclohexene derivatives, such as *d*-limonene, administered orally, are absorbed and distributed throughout the body. Following oral administration to humans, *d*-limonene was distributed preferentially to fatty tissues, as indicated by a high oil-blood partition coefficient and a long half-life during the slow elimination phase. Because of the limited molecular weight of the candidate substances in this group (range ~ 136-204 D) and their lipophilic character (e.g. estimated or measured log Kow values for β-phellandrene, β-bisabolene and *d*-limonene are approximately 4.7, 7.1 or 4.6, respectively) it may be assumed that all of the substances in this group may be absorbed, although the extent of absorption for individual substances cannot be accurately estimated from these physico-chemical properties.

*Subgroup IV*

The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.

*Subgroup V*

For the substances in subgroup V (bicyclic, non-aromatic hydrocarbons), the available data from oral studies are incomplete. These studies have only addressed the excretion of some supporting and longifolene [former FL-no: 01.047] in the form of metabolites via the urine. No mass-balance data were provided. So, these studies only show that these substances will be absorbed to some extent. The same may be anticipated for the other candidate substances in this subgroup. For the supporting substances α- and β-pinene and delta-carene information on kinetics is available from humans exposed via inhalation, in occupational settings. These studies show that these substances can be absorbed after inhalation exposure and that metabolites will be excreted into the urine e.g. as glucuronide conjugates. The elimination follows a triphasic pattern with rather long terminal half-lives and the absorbed amount will be eliminated within several days. Based on the lipophilic character of these substances it may be anticipated that they will preferentially distribute in the adipose tissues, which is supported by the slow terminal elimination rates.

*Subgroup VI*

The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.

**Metabolism**

*Subgroup I*

The substances in subgroup I can be expected to be metabolised through omega-oxidation which will lead to the formation of alcohols, and after subsequent further oxidation to carboxylic acids, which may be further oxidised via β-oxidation to yield carbon dioxide. Extensive mass balance data are not available, but in some of the available studies excretion of carboxylic acids and carbon dioxide has been reported. Formation of keto-acids has also been reported, and these may be thought to be formed from omega-oxidation at one end of the molecule and omega-1 or -2 oxidation at the other end. More interest has been put in the identification of metabolites, which did not undergo substantial chain shortening. These metabolites will arise from oxidation of the non-terminal carbon atoms, e.g. via omega-1, -2, -3 or even -4 oxidation. The resulting secondary alcohols can be conjugated with e.g. glucuronic acid and excreted via the urine, or can be further oxidised to yield ketones. A subsequent introduction of another secondary hydroxyl group may result in the formation of diketones. With n-hexane, the resulting 2,5-hexanedione (a gamma-diketone) has been demonstrated to be responsible for hexane-induced neurotoxicity. Neurotoxicity is a common feature of gamma-diketones, and for that reason, candidate substance 3-methylhexane [FL-no: 01.050] cannot be anticipated to be metabolised into innocuous compounds. In addition, it is known that methyl-branching of the carbon chain potentiates the neurotoxicity of the gamma-diketone (Topping et al., 1994; EFSA, 2004b). The two other hexane derivatives in this group [FL-no: 01.033 and 01.034] cannot be oxidised to give a gamma-diketone, due to the presence of methyl groups on the C2 and C5 carbon atoms. Therefore, these substances may be considered as being metabolised to innocuous metabolites. For the longer chain alkanes in this group it would require oxidation of the more central carbon atoms in order to be converted into gamma-diketones. Such oxidations are less favourable than omega, omega-1 or -2 oxidations and therefore it is concluded that oxidation of these higher alkanes will not result in toxicologically relevant levels of gamma-diketones, also because of rapid conjugation of the precursor alcohols. For heptane it was shown that this substance does not result in neurotoxicity (Bahima et al., 1984), although the gamma-diketone itself (2,5-heptanedione) is known to be neurotoxic (Topping et al., 1994). The remaining three candidate flavouring substances in this group (i.e. [FL-no: 01.038, 01.054 and 01.057]) can be expected to be metabolised to innocuous products.

*Subgroup II*

The data available on the metabolism of one supporting and one structurally related substance in subgroup II show that metabolic options for the chemicals in this group are epoxidation of double bonds, ultimately resulting in diols, which can be further conjugated. With the supporting substance myrcene, further metabolism of the diols into carboxylic acids has also been reported. Both in rats and rabbits, the principal urinary metabolite following gavage administration of myrcene is myrcene-3,10-glycol, formed from the hydration of the epoxide intermediate in both species. Epoxidation of the 3,10-double bond was favoured over epoxidation of the 1,2-double bond.

The studies indicate that the formation of diols from the myrcene-epoxides is very efficient. It is noted, however, that the diols and the related carboxylic acids are all the result of epoxidation of double bonds in which one of the carbon atoms has only hydrogen substituents, but no further carbon chains. In this respect, the candidate substance cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] is most similar to myrcene. With myrcene no diepoxide metabolites or further reaction products thereof have been reported, but it is noted that mass balance data are highly incomplete, so some metabolites may have been overlooked.

With the structurally related substance, 2-methyl-buta-1,3-diene, epoxidation of the 1,2-double bond is favoured over epoxidation of the 3,4-double bond. While the 1,2-epoxide is readily hydrolysed, the 3,4-epoxide is far more stable. Both metabolites can be converted to the corresponding diols and at least one hydroxy-carboxylic acid (2-hydroxy-2-methyl-3-butenoic acid) has been identified. In addition, in particular the 3,4-epoxide metabolite can be oxidised for a second time, resulting in the formation of a diepoxide metabolite, which is known to be reactive and has been suggested to be responsible for 2-methylbuta-1,3-diene-induced DNA damage.

Apart from myrcene and 2-methylbuta-1,3-diene, terminal double bonds appear in candidate substances [FL-no: 01.064 and 01.070]. In contrast to corresponding substances in FGE.07 and FGE.18 there are no other functional groups in the molecule (e.g. hydroxyl- or keto-group) that could provide a more direct option for detoxication e.g. via conjugation with glucuronide or sulphate. In the two FGEs mentioned above, the presence of such groups was an argument to conclude that the metabolism of the particular candidate substances would give rise to innocuous products. It would not be possible to conclude similarly for the candidate substances in FGE.25. The other candidate substances in this subgroup (II) might also be oxidised in the various methyl groups but no data are available to substantiate this. Because of these considerations, it cannot be concluded that the candidate substances in this subgroup [FL-no: 01.035, 01.064 and 01.070] will be metabolised to innocuous products.

*Subgroup III*

Hardly any data are available on the metabolism of the candidate substances in subgroup III. Only for *l*-limonene a very limited amount of data was available on biotransformation in incubations with rat liver microsomes. In comparison with the structures of the candidate substances in subgroup III, the structure of the one supporting chemical *d*-limonene is comparatively simple. Major differences between *d*-limonene and the candidate substances are the length of the ring substituents and the number of double bonds.

Allylic oxidation is by far the major pathway for metabolism of limonene in humans. Minor pathways in limonene metabolism reported for the rat include epoxidation of either the 1,2- or the 8,9-double bond and subsequent hydrolysis to the diol. Given the many metabolic options for this substance, a myriad of metabolites has been found, including conjugates, and for a change mass balance data are available, which show that in various animal species the substance is completely eliminated within three days, predominantly via the urine. No genotoxicity of limonene epoxides could be detected, and it may well be concluded that *d*-limonene is metabolised to innocuous substances. However, although it is very likely that the candidate substances may undergo the same metabolic conversions (allylic oxidations and double bond epoxidation) the conclusion for *d*-limonene cannot be extrapolated to all of the candidate substances because of the structural dissimilarities and the absence of any further data on molecules with closer resemblance to the candidate substances. The data available for *l*-limonene do not indicate that major differences are to be anticipated between the metabolism of *d*-limonene and *l*-limonene. For bisabola-1,8,12-triene [FL-no: 01.027], β-bisabolene [FL-no: 01.028] and delta-elemene [FL-no: 01.039] it may be concluded that these are metabolised to innocuous products.

It is noted that *d*-limonene (metabolites) causes α2μ-microglobulin accumulation in male kidneys, an event known to be associated with male rat specific nephropathy[21], and irrelevant for human toxicological risk assessment.

*Subgroup IV*

The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.

[21] For introduction into this area see e.g. the referred paper by Lehman-McKeeman *et al*. (1989)

*Subgroup V*

Metabolism data for subgroup V are available for the supporting substances pinenes, camphene, caryophyllene, delta-3-carene, pinane and carane. In general the metabolic options for these substances include oxidation of methyl ring substituent groups to give the corresponding alcohol and further oxidation products. For the substances studied, double bond epoxidation has also been demonstrated. In addition, ring cleavage has also been observed, e.g. for β-pinene resulting in the formation of monocyclic terpenoid derivatives like α-terpineol. Hydroxylated metabolites or further metabolised products may be eliminated as conjugates e.g. with glucuronic acid. However, given the lack of data on the candidate substances it cannot be concluded that the candidate substance in this subgroup [FL-no: 01.059] can be metabolised to innocuous products.

Based on similarity with β-pinene it may be speculated that the candidate substance 4(10)-thujene [FL-no: 01.059] may be hydroxylated to thujyl alcohol, which is known to be conjugated with glucuronic acid and eliminated via urine (Hämäläinen, 1912; EFSA, 2009a). However, based on the same similarity, epoxidation of the exocyclic double bond may also be expected, and it is not clear what other reactions might occur. No indications of the relevance of these various routes is available. Hence, despite the knowledge of the fate of thujyl alcohol, also for thujene it cannot be concluded that it will be metabolised to innocuous products.

*Subgroup VI*

The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.

### C.6. Overall Conclusion on Absorption, Distribution, Metabolism and Elimination.

Generally, the available data indicate that the aliphatic and aromatic hydrocarbons participate in similar pathways of metabolic detoxication. Being lipophilic and of low molecular weight, these hydrocarbons may be assumed to be absorbed by the gastrointestinal tract. Subsequently, they are oxidised to more polar oxygenated metabolites e.g. by CYP-450 enzymes. The phase I metabolites are then conjugated and excreted mainly in the urine. The candidate substances and supporting substances are oxidised either by side chain oxidation or epoxidation of the exocyclic or endocyclic double bond. Oxidation initially yields hydroxylated metabolites that may be excreted in conjugated form or undergo further oxidation, yielding more polar metabolites that are also excreted. If a double bond is present, epoxide metabolites may form that are further metabolised either by hydrolysis to yield diols or by conjugation with glutathione to yield mercapturic acid derivatives. The saturated alkanes in this group may be metabolised via omega and omega-1, -2, -3 or -4 oxidation. Whereas omega oxidation would ultimately lead to the formation of carboxylic acids, the other oxidations would give rise to secondary alcohols and ketones. The carboxylic acids may be expected to participate in the endogenous fatty acid metabolism. However, for most of the subgroups the information was incomplete and the similarity between supporting and candidate substances was limited. In addition, proper mass balance data were not available. Some mass balance data available indicated slow elimination. For several subgroups no data were available at all. In Table C.2 the final conclusions for each of the candidate substances has been presented, together with a brief explanatory statement, about the conclusion reached. It is noted that the subgroup III supporting substance *d*-limonene causes α2μ-microglobulin accumulation in male kidneys, an event known to be associated with male rat specific nephropathy, and irrelevant for human toxicological risk assessment.

**Table C.2** Can innocuous metabolites be expected to be formed based on available data?

| FL-no: | Substance name | Innocuous metabolites? |
|---|---|---|
| **Subgroup I: ACYCLIC ALKANES** | | |
| 01.033 | 2,2-Dimethylhexane | Yes |
| 01.034 | 2,4-Dimethylhexane | Yes |
| 01.038 | Dodecane | Yes |
| 01.054 | Pentadecane | Yes |
| 01.057 | Tetradecane | Yes |
| **Subgroup II: ACYCLIC ALKENES** | | |
| 01.035 | 2,6-Dimethylocta-2,4,6-triene | No (lack of supporting data) |
| Deleted from the Register | 2-Methylbuta-1,3-diene | No (known biotransformation to reactive metabolite responsible for toxicity and genotoxicity) |
| 01.064 | cis-3,7-Dimethyl-1,3,6-octatriene | No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options) |
| 01.070 | 1-Octene | No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options) |
| **Subgroup III: CYCLOHEXENE HYDROCARBONS** | | |
| 01.027 | Bisabola-1,8,12-triene | Yes |
| 01.028 | β-Bisabolene | Yes |
| 01.039 | delta-Elemene | Yes |
| 01.001 | Limonene | Yes |
| 01.046 | l-Limonene | Yes |
| **Subgroup IV: AROMATIC HYDROCARBONS** | | |
| The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry. | | |
| **Subgroup V: BICYCLIC, NON-AROMATIC HYDROCARBONS** | | |
| 01.059 | 4(10)-Thujene | No (but supported by the supporting substance β-caryophyllene [FL-no: 01.007]) |
| **Subgroup VI: MACROCYCLIC, NON-AROMATIC HYDROCARBONS** | | |
| The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry. | | |

## Appendix D. Substances no Longer Supported for Use as Flavouring Substances in Europe by Industry

Since the publication of FGE.25Rev2 (EFSA CEF Panel, 2011), the following 23 substances [former FL-no: 01.021, 01.022, 01.023, 01.030, 01.031, 01.032, 01.036, 01.037, 01.042, 01.043, 01.044, 01.047, 01.050, 01.051, 01.052, 01.053, 01.055, 01.056, 01.058, 01.060, 01.066, 01.067 and 01.078] of 37 candidate substances evaluated in FGE.25Rev2 are no longer supported for use as flavouring substances in Europe by Industry and will therefore not be considered any further (DG SANCO, 2012).

The 23 substances were evaluated via the B-side of the Procedure scheme and as they all had estimated European daily *per capita* intakes below the threshold of concern for their respective structural class they all proceeded to step B4 of the Procedure. Here the Panel concluded that additional data were required for these substances as no NOAELs were available for any of these substances or for any structurally related substances. The 23 substances are listed here below together with their specifications.

**Table D1:** Specification Summary of the Substances no Longer Supported by Industry

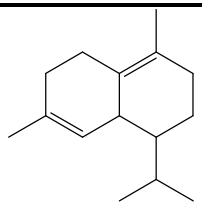
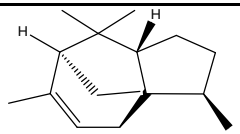
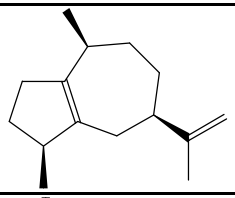
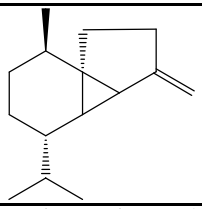
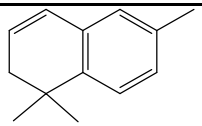
| *Former FL-no* | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight | Solubility[a]<br>Solubility in ethanol[b] | Boiling point, °C[c]<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index[d]<br>Spec.gravity[e] | Specification comments |
|---|---|---|---|---|---|---|---|---|
| *01.021* | delta-Cadinene |  | 10982<br>29350-73-0 | Liquid<br>$C_{15}H_{24}$<br>204.36 | Freely soluble | 286<br><br>MS<br>95 % | 1.497-1.503<br>0.917-0.923 | No longer supported by Industry (DG SANCO, 2012). |
| *01.022* | α-Cedrene |  | 10985<br>469-61-4 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 119 (13 hPa)<br><br>MS<br>95 % | 1.500-1.506<br>0.932-0.938 | No longer supported by Industry (DG SANCO, 2012). |
| *01.023* | 1(5),11-Guaiadiene |  | 11003<br>3691-12-1 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 78 (3 hPa)<br><br>MS<br>95 % | 1.493-1.499<br>0.897-0.903 | No longer supported by Industry (DG SANCO, 2012). |
| *01.030* | β-Cubebene |  | 10989<br>13744-15-5 | Solid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 284<br>60<br>MS<br>95 % | n.a.<br>n.a. | No longer supported by Industry (DG SANCO, 2012). |
| *01.031* | 1,2-Dihydro-1,1,6-trimethylnaphthalene |  | 30364-38-6 | Liquid<br>$C_{13}H_{16}$<br>172.27 | Practically insoluble or insoluble<br>Freely soluble | 115 (24 hPa)<br><br>MS<br>95 % | 1.542-1.548<br>0.942-0.948 | No longer supported by Industry (DG SANCO, 2012). |

**Table D1:** Specification Summary of the Substances no Longer Supported by Industry

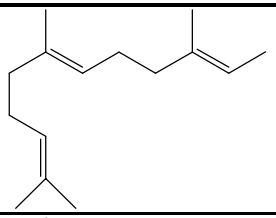
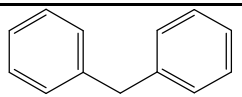
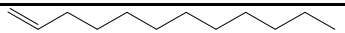
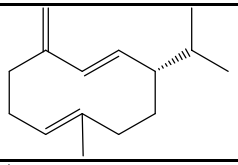
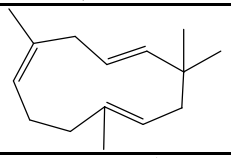
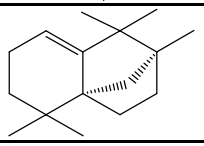
| *Former FL-no* | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight | Solubility[(a)]<br>Solubility in ethanol[(b)] | Boiling point, °C[(c)]<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index[(d)]<br>Spec.gravity[(e)] | Specification comments |
|---|---|---|---|---|---|---|---|---|
| *01.032* | 2,3-Dihydrofarnesene |  | 7681-88-1 | Liquid<br>$C_{15}H_{26}$<br>206.37 | Practically insoluble or insoluble<br>Freely soluble | 130 (15 hPa)<br><br>NMR<br>95 % | 1.468-1.474<br>0.817-0.823 | No longer supported by Industry (DG SANCO, 2012). |
| *01.036* | Diphenylmethane |  | 11847<br>101-81-5 | Solid<br>$C_{13}H_{12}$<br>168.24 | Practically insoluble or insoluble<br>Freely soluble | 262<br>27<br>MS<br>95 % | n.a.<br>n.a. | No longer supported by Industry (DG SANCO, 2012). |
| *01.037* | Dodec-1-ene |  | 10992<br>112-41-4 | Liquid<br>$C_{12}H_{24}$<br>168.23 | Practically insoluble or insoluble<br>Freely soluble | 213<br><br>MS<br>95 % | 1.425-1.431<br>0.755-0.761 | No longer supported by Industry (DG SANCO, 2012). |
| *01.042* | Germacra-1(10),4(14),5-triene |  | 23986-74-5 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 308<br><br>MS<br>95 % | 1.507-1.513<br>0.896-0.892 | No longer supported by Industry (DG SANCO, 2012). |
| *01.043* | 3,7,10-Humulatriene |  | 11004<br>6753-98-6 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 123 (13 hPa)<br><br>MS<br>95 % | 1.499-1.505<br>0.889-0.895 | No longer supported by Industry (DG SANCO, 2012). |
| *01.044* | Isolongifolene |  | 1135-66-6 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 120 (16 hPa)<br><br>MS<br>95 % | 1.495-1.501<br>0.926-0.932 | No longer supported by Industry (DG SANCO, 2012). |

**Table D1:** Specification Summary of the Substances no Longer Supported by Industry

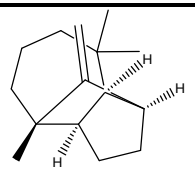
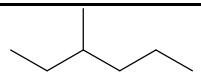
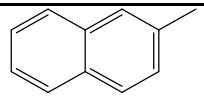
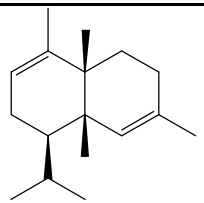
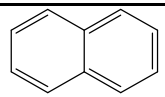
| *Former FL-no* | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight | Solubility[a]<br>Solubility in ethanol[b] | Boiling point, °C[c]<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index[d]<br>Spec.gravity[e] | Specification comments |
|---|---|---|---|---|---|---|---|---|
| *01.047* | Longifolene |  | 475-20-7 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 115 (13 hPa)<br><br>MS<br>95 % | 1.498-1.504<br>0.929-0.935 | No longer supported by Industry (DG SANCO, 2012). |
| *01.050* | 3-Methylhexane |  | 589-34-4 | Liquid<br>$C_7H_{16}$<br>100.20 | Practically insoluble or insoluble<br>Freely soluble | 92<br><br>MS<br>95 % | 1.385-1.391<br>0.684-0.690 | No longer supported by Industry (DG SANCO, 2012). |
| *01.051* | 2-Methylnaphthalene |  | 11010<br>91-57-6 | Solid<br>$C_{11}H_{10}$<br>142.20 | Practically insoluble or insoluble<br>Freely soluble | 241<br>35<br>MS<br>95 % | n.a.<br>n.a. | No longer supported by Industry (DG SANCO, 2012). |
| *01.052* | α-Muurolene |  | 11011<br>10208-80-7 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 118 (17 hPa)<br><br>MS<br>95 % | 1.502-1.508<br>0.911-0.917 | No longer supported by Industry (DG SANCO, 2012). |
| *01.053* | Naphthalene |  | 11014<br>91-20-3 | Solid<br>$C_{10}H_8$<br>128.17 | Practically insoluble or insoluble<br>Freely soluble | 218<br>80<br>MS<br>95 % | n.a.<br>n.a. | No longer supported by Industry (DG SANCO, 2012). |

**Table D1:** Specification Summary of the Substances no Longer Supported by Industry

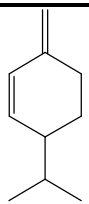
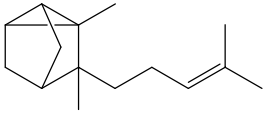
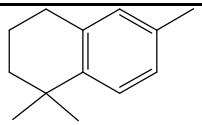
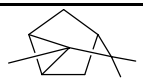
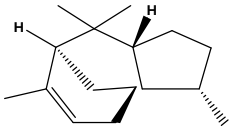
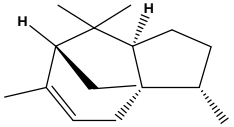
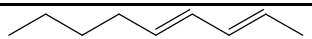
| *Former FL-no* | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight | Solubility[(a)]<br>Solubility in ethanol[(b)] | Boiling point, °C[(c)]<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index[(d)]<br>Spec.gravity[(e)] | Specification comments |
|---|---|---|---|---|---|---|---|---|
| *01.055* | β-Phellandrene |  | 11017<br>555-10-2 | Liquid<br>$C_{10}H_{16}$<br>136.24 | Practically insoluble or insoluble<br>Freely soluble | 174<br><br>MS<br>95 % | 1.476-1.482<br>0.839-0.845 | No longer supported by Industry (DG SANCO, 2012). |
| *01.056* | α-Santalene |  | 512-61-8 | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 112 (9 hPa)<br><br>MS<br>95 % | 1.480-1.486<br>0.895-0.901 | No longer supported by Industry (DG SANCO, 2012). |
| *01.058* | 1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene |  | 475-03-6 | Liquid<br>$C_{13}H_{18}$<br>174.29 | Practically insoluble or insoluble<br>Freely soluble | 245<br><br>MS<br>95 % | 1.519-1.525<br>0.932-0.938 | No longer supported by Industry (DG SANCO, 2012). |
| *01.060* | 1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane |  | 508-32-7 | Solid<br>$C_{10}H_{16}$<br>136.24 | Practically insoluble or insoluble<br>Freely soluble | 152<br>66<br>MS<br>95 % | n.a.<br>n.a. | No longer supported by Industry (DG SANCO, 2012). |
| *01.066* | 2-Cedrene |  | | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 82 (11 hPa)<br><br>MS<br>95 % | 1.495-1.501<br>0.928-0.934 | No longer supported by Industry (DG SANCO, 2012). |
| *01.067* | 8(14)-Cedrene |  | | Liquid<br>$C_{15}H_{24}$<br>204.35 | Practically insoluble or insoluble<br>Freely soluble | 118 (13 hPa)<br><br>MS<br>95 % | 1.498-1.504<br>0.930-0.936 | No longer supported by Industry (DG SANCO, 2012). |
| *01.078* | 2,4-Nonadiene |  | 4292<br><br>56700-78-8 | Liquid<br>$C_9H_{16}$<br>124.23 | Insoluble<br>Slightly soluble | 155<br><br>MS | 1.446<br>0.755 | No longer supported by |

**Table D1:** Specification Summary of the Substances no Longer Supported by Industry

| *Former FL-no* | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight | Solubility[(a)]<br>Solubility in ethanol[(b)] | Boiling point, °C[(c)]<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index[(d)]<br>Spec.gravity[(e)] | Specification comments |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 79 % | | Industry (DG SANCO, 2012). |

(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1013.25 hPa, if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.
n.a. Not applicable

The candidate substances in FGE.25Rev2 were allocated to subgroups. The subgrouping of the 23 substances no longer supported by Industry is shown here (Table D2). Subgroups still remaining in FGE.25Rev3 are: I, II, III and V. The Subgroups IVa, IVb, IVc and VI no longer contain any substances.

**Table D2:** Subgroups of Substances no longer Supported by Industry.

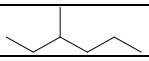
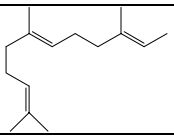
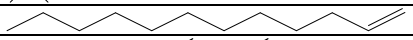
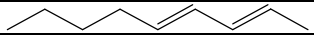
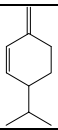
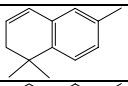
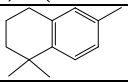
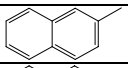
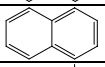
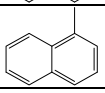
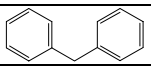
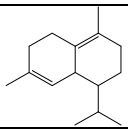
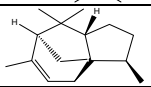
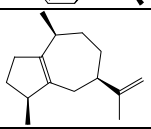
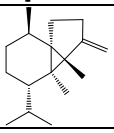
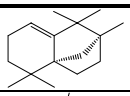
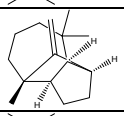
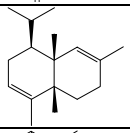
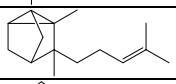
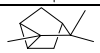
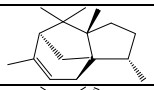
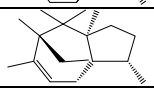
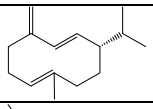
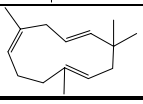
| ***Former FL-no*** | **Name** | **Structural formula** | **Structural class** |
|---|---|---|---|
| **I: ACYCLIC ALKANES** | | | |
| *01.050* | 3-Methylhexane |  | I |
| **II: ACYCLIC ALKENES** | | | |
| *01.032* | 2,3-Dihydrofarnesene |  | I |
| *01.037* | Dodec-1-ene |  | I |
| *01.078* | 2,4-Nonadiene |  | I |
| **III: CYCLOHEXENE HYDROCARBONS** | | | |
| *01.055* | β-Phellandrene |  | I |
| **IV: AROMATIC HYDROCARBONS** | | | |
| **IVa: BENZENE HYDROCARBONS** | | | |
| *01.031* | 1,2-Dihydro-1,1,6-trimethylnaphthalene |  | I |
| *01.058* | 1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene |  | II |
| **IVb: NAPHTHALENE HYDROCARBONS** | | | |
| *01.051* | 2-Methylnaphthalene |  | III |
| *01.053* | Naphthalene |  | III |
| (01.014) | (1-Methylnaphthalene) |  | III |
| **IVc: DIPHENYLMETHANE** | | | |
| *01.036* | Diphenylmethane |  | III |
| **V: BI- and TRICYCLIC, NON-AROMATIC HYDROCARBONS** | | | |
| *01.021* | Delta-Cadinene |  | III |
| *01.022* | α-Cedrene |  | I |
| *01.023* | 1(5),11-Guaiadiene |  | I |
| *01.030* | β-Cubebene |  | I |

**Table D2:** Subgroups of Substances no longer Supported by Industry.

| ***Former FL-no*** | **Name** | **Structural formula** | **Structural class** |
|---|---|---|---|
| *01.044* | Isolongifolene |  | I |
| *01.047* | Longifolene |  | I |
| *01.052* | α-Muurolene |  | I |
| *01.056* | α-Santalene |  | I |
| *01.060* | 1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane |  | I |
| *01.066* | 2-Cedrene |  | I |
| *01.067* | 8(14)-Cedrene |  | I |
| **VI: MACROCYCLIC, NON-AROMATIC HYDROCARBONS** | | | |
| *01.042* | Germacra-1(10),4(14),5-triene |  | I |
| *01.043* | 3,7,10-Humulatriene |  | I |

## ABBREVIATIONS

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| BW | Body weight |
| CAS | Chemical Abstract Service |
| CEF | Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CHO | Chinese hamster ovary (cells) |
| CoE | Council of Europe |
| CPN | Chronic progressive nephropathy |
| DNA | Deoxyribonucleic acid |
| DTU-NFI | Danish Technical University – National Food Institute |
| EC | European Commission |
| EFFA | European Flavour and Fragrance Association |
| EPA | United States Environmental Protection Agency |
| EU | European Union |
| FAO | Food and Agriculture Organization of the United Nations |
| FEMA | Flavor and Extract Manufacturers Association |
| FGE | Flavouring Group Evaluation |
| FLAVIS (FL) | Flavour Information System (database) |
| GLP | Good laboratory practise |
| ID | Identity |
| IOFI | International Organization of the Flavor Industry |
| Ip | Intraperitoneal |
| IR | Infrared spectroscopy |
| ISS | Istituto Superiore di Sanita |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LOAEL | Lowest Observed Adverse Effect Level |
| MSDI | Maximised Survey-derived Daily Intake |
| mTAMDI | Modified Theoretical Added Maximum Daily Intake |

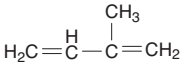
| | |
|---|---|
| NCE | Normochromatic erythrocyte |
| NOAEL | No observed adverse effect level |
| NTP | National Toxicology Program |
| OECD | Organisation for Economic Co-operation and Development |
| PCE | Polychromatic erythrocyte |
| SCE | Sister chromatic exchange |
| SCF | Scientific Committee on Food |
| UDS | Unscheduled DNA Synthesis |
| US EPA | United States Environmental Protection Agency |
| WHO | World Health Organization |

# Isoprene

## CAS No. 78-79-5

Reasonably anticipated to be a human carcinogen

First listed in the *Ninth Report on Carcinogens* (2000)



## Carcinogenicity

Isoprene is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals.

### *Cancer Studies in Experimental Animals*

Exposure to isoprene by inhalation caused tumors at several different tissue sites in mice and rats. In mice of both sexes, isoprene caused blood-vessel cancer (hemangiosarcoma) and benign or malignant tumors of the Harderian gland (adenoma or carcinoma) and the lung (alveolar/bronchiolar adenoma or carcinoma). In male mice, it also caused cancer of the hematopoietic system (histiocytic sarcoma) and benign or malignant tumors of the liver (hepatocellular adenoma or carcinoma) and forestomach (squamous-cell papilloma or carcinoma). In rats of both sexes, isoprene caused benign or malignant tumors of the mammary gland (fibroadenoma or carcinoma) and kidney (renal-cell adenoma or carcinoma). In male rats, it also caused benign tumors of the testis (adenoma) (NTP 1995, Placke *et al.* 1996, Melnick and Sills 2001).

### *Studies on Mechanisms of Carcinogenesis*

Isoprene is the 2-methyl analogue of 1,3-butadiene, an industrial chemical that has been identified as a carcinogen in humans and experimental animals (Gervasi *et al.* 1985, NTP 1999a,b). The isoprene analogue isopentenyl pyrophosphate is a building block of cholesterol synthesis, and levels of exhaled isoprene correlate with cholesterol synthesis (IARC 1994, Rieder *et al.* 2001). Isoprene and butadiene are metabolized to monoepoxide and diepoxide intermediates by liver microsomal cytochrome P450-dependent monooxygenases from several species, including humans (Gervasi *et al.* 1985, IARC 1994, NTP 1999a). These intermediates may be detoxified by hydrolysis (catalyzed by epoxide hydrolase) or conjugation with glutathione (catalyzed by glutathione S-transferase).

The diepoxide intermediates of isoprene and butadiene caused mutations in *Salmonella typhimurium*, whereas the monoepoxides of isoprene and parent compounds did not. In mammalian cells *in vitro,* isoprene did not cause sister chromatid exchange, chromosomal aberrations, or micronucleus formation (NTP 1995, 1999a), but did cause DNA damage in human peripheral-blood mononuclear cells and human leukemia cells when incubated with microsomal enzymes (Fabiani *et al.* 2007). In mice exposed *in vivo,* isoprene and 1,3-butadiene caused sister chromatid exchange in bone-marrow cells and micronucleus formation in peripheral-blood erythrocytes (Tice 1988, Tice *et al.* 1988). Sites at which both isoprene and butadiene caused tumors in rodents include the liver, lung, Harderian gland, forestomach, hematopoietic tissue, and circulatory system in mice and the mammary gland, kidney, and testis in rats (NTP 1999a,b). Harderian-gland tumors caused by isoprene in mice had a high frequency of unique mutations of the K-*ras* protooncogene (A to T transversions at codon 61) (Hong *et al.* 1997).

There is no evidence to suggest that mechanisms by which isoprene causes tumors in experimental animals would not also operate in humans.

### *Cancer Studies in Humans*

No epidemiological studies were identified that evaluated the relationship between human cancer and exposure specifically to isoprene.

## Properties

Isoprene is structurally similar to 1,3-butadiene and exists as a colorless, volatile liquid at room temperature (NTP 1999a). It occurs frequently in nature and is emitted to the environment by plants and trees. Isoprene is practically insoluble in water, but is soluble in ethanol, diethyl ether, benzene, and acetone. It is stable under normal conditions, but it is very flammable and will polymerize vigorously or decompose with abrupt changes in temperature or pressure (IARC 1994, Akron 2009). Physical and chemical properties of isoprene are listed in the following table.

| Property | Information |
|---|---|
| Molecular weight | 68.1 |
| Specific gravity | 0.681 at 20°C/4°C |
| Melting point | –145.95°C |
| Boiling point | 34.07°C at 760 mm Hg |
| Log $K_{ow}$ | 2.42 |
| Water solubility | 0.642 g/L at 25°C |
| Vapor pressure | 550 mm Hg at 25°C |
| Vapor density relative to air | 2.4 |

Source: HSDB 2009.

## Use

The majority of isoprene produced commercially is used to make synthetic rubber (*cis*-polyisoprene), most of which is used to produce vehicle tires. The second- and third-largest uses are in the production of styrene-isoprene-styrene block polymers and butyl rubber (isobutene-isoprene copolymer) (IARC 1994).

## Production

Isoprene is recovered as a by-product of thermal cracking of naphtha or gas oil from $C_5$ streams (IARC 1994, NTP 1999a). The isoprene yield is about 2% to 5% of the ethylene yield. U.S. demand for isoprene grew 6.5% annually from 1985 to 1992 (NTP 1999a). In 1994, isoprene production in the United States was about 619 million pounds, almost 29% more than in 1992. Estimated isoprene production capacity for eight facilities was 598 million pounds in 1996, based on estimates of isoprene content of product stream available from ethylene production via heavy liquids. In 2009, isoprene was produced by 22 manufacturers worldwide, including 12 U.S. producers (SRI 2009), and was available from 23 suppliers, including 12 U.S. suppliers (ChemSources 2009). U.S. imports of isoprene (purity ≥ 95% by weight) increased from zero in 1989 to a peak of 144 million pounds in 2003. Imports declined to 19.6 million pounds in 2004, the lowest level since 1992, but remained near 32 million pounds from 2005 through 2008. During this period, U.S. exports of isoprene ranged from 7.9 million to 39.6 million pounds (in 2006) (USITC 2009). Reports filed from 1986 to 2002 under the U.S. Environmental Protection Agency's Toxic Substances Control Act Inventory Update Rule indicated that U.S. production plus imports of isoprene totaled 100 million to 500 million pounds (EPA 2004).

## Exposure

Isoprene is formed endogenously in humans at a rate of 0.15 µmol/kg of body weight per hour, equivalent to approximately 2 to 4 mg/kg per

day (Taalman 1996), and is the major hydrocarbon in human breath (accounting for up to 70% of exhaled hydrocarbons) (Gelmont *et al.* 1981). Concentrations in human blood range from 1.0 to 4.8 µg/L (Cailleux *et al.* 1992). Isoprene is produced at higher rates in males than females. The rate of isoprene production increases with age up to the age of 29 (Lechner *et al.* 2006); it is lower in young children than adults by a factor of about 2.4 (Taucher *et al.* 1997). In a study of 30 adult volunteers, the mean isoprene concentration measured in alveolar breath was 118 ppb, with a range of 0 to 474 ppb (Turner *et al.* 2006). After 20 to 30 minutes of exercise, isoprene concentration in exhaled air decreased to a range of 0 to 40 ppb (Senthilmohan *et al.* 2000). Smoking one cigarette increased the concentration of isoprene in exhaled air by 70% (Senthilmohan *et al.* 2001). Isoprene is also produced endogenously by other animals. Production rates reported for rats and mice were 1.9 and 0.4 µmol/kg of body weight per hour, respectively (Peter *et al.* 1987).

Foods of plant origin would be expected to be a source of daily exposure to isoprene, since isoprene is emitted by agricultural crops and is the basic structural unit in countless natural products found in foods, such as terpenes and vitamins A and K (NTP 1999a). Isoprene has been reported to occur in the essential oil of oranges, the fruit of hops, carrot roots, and roasted coffee (Taalman 1996, NTP 1999a).

Isoprene is emitted from plants and trees and is present in the general environment at low concentrations (Taalman 1996). Isoprene emissions from many types of plants have been estimated under various climatic conditions, to evaluate their importance in global climate change (Mayrhofer *et al.* 2004, Parra *et al.* 2004, Schnitzler *et al.* 2004, 2005, Pegoraro *et al.* 2005, Sasaki *et al.* 2005, Sharkey 2005, Moukhtar *et al.* 2006, Simon *et al.* 2006, Tambunan *et al.* 2006). Annual global isoprene emissions, estimated at 175 billion to 503 billion kilograms (386 billion to 1,109 billion pounds), account for an estimated 57% of total global natural volatile organic compound emissions (Guenther *et al.* 1995). The average biogenic emission rate factor for isoprene in U.S. woodlands is 3 mg/m$^2$ per hour (compared with 5.1 mg/m$^2$ for total volatile organic compounds) (Guenther *et al.* 1994). Isoprene concentrations in biogenic emissions range from 8% to 91% of total volatile organic compounds, averaging 58%. Because isoprene biosynthesis is associated with photosynthesis, isoprene emissions are negligible at night (Lamb *et al.* 1993). Because isoprene is emitted primarily by deciduous trees, emissions are seasonal, being highest in the summer and lowest in the winter (Guenther *et al.* 1994, Fuentes and Wang 1999). The south central and southeastern areas of the United States have the highest biogenic emissions (Lamb *et al.* 1993, Guenther *et al.* 1994). The half-life of atmospheric isoprene has been estimated at 0.5 hours by reaction with nitric oxide, 4 hours by reaction with hydroxyl radicals, and 19 hours by reaction with ozone (HSDB 2009).

Anthropogenic sources of isoprene in the atmosphere include ethylene production by cracking naphtha, wood pulping, oil fires, wood-burning stoves and fireplaces, other biomass combustion, tobacco smoking (200 to 400 µg per cigarette), gasoline, and exhaust from turbines and automobiles (Adam *et al.* 2006, HSDB 2009). Isoprene has been measured as one of the volatile organic compounds in the ambient air in regions with industrial pollution, and in urban, residential, and rural areas as an indicator of the potential for ozone formation. Thus, isoprene is a key indicator for regional air quality, as well as being a component of the global carbon cycle (Borbon *et al.* 2004, Guo *et al.* 2004, Kuster *et al.* 2004, Warneke *et al.* 2005, Hellen *et al.* 2006).

The reported concentration of isoprene in U.S. ambient air ranges from 1 to 21 parts per billion carbon (ppbC) and generally is less than 10 ppbC. Isoprene accounts for less than 10% of non-methane hydrocarbons in ambient air. Biogenic hydrocarbons may contribute more to total atmospheric hydrocarbons under stagnant atmospheric conditions (Altschuller 1983, Hagerman *et al.* 1997). The major sources of isoprene in ambient air appear to be biogenic emissions at rural sites and vehicular emissions in urban areas (Borbon *et al.* 2001, So and Wang 2004). Where the source is primarily biogenic, the isoprene concentration slowly increases during the day, reaching a peak in the middle of the day, when photosynthesis is greatest. Where vehicular emissions are the primary source, the isoprene concentration peaks during the morning and evening rush hours and is low in the middle of the day (Borbon *et al.* 2002). One study concluded that in summer, at least 80% of the isoprene at a rural site was due to biogenic emissions, but that in winter, more than 90% of residual isoprene was from urban air-mass mixing (Borbon *et al.* 2004). Where industrial emissions are the primary source of isoprene, the concentration may peak at night, or there may be no peak at all (Zhao *et al.* 2004, Chiang *et al.* 2007).

The primary source of isoprene in indoor air is environmental tobacco smoke. Isoprene was found to be the major component of hydrocarbons in the air of a smoky café (10 patrons smoking, 10 not smoking) (16.7%) and in sidestream smoke (29.2%) (Barrefors and Petersson 1993). A monitoring survey in November 1992 in homes and workplaces in the greater Philadelphia area found mean isoprene concentrations in personal air samples of 4.65 µg/m$^3$ in 60 nonsmoking homes, 18.15 µg/m$^3$ in 29 homes with smokers, 5.29 µg/m$^3$ in 51 nonsmoking workplaces, and 22.80 µg/m$^3$ in 28 workplaces that allowed smoking (Heavner 1996). A survey in the Lower Rio Grande Valley of Texas reported a median summertime isoprene concentration of 2.90 µg/m$^3$ for three indoor air samples (it was not reported whether the occupants were smokers or nonsmokers), compared with 0.40 µg/m$^3$ for three outdoor air samples (Mukerjee 1997).

Air-monitoring data were collected at three U.S. facilities that produced isoprene monomers or polymers; 98.5% of the samples showed concentrations of less than 10 ppm, and 91.3% of less than 1 ppm (Leber 2001, Lynch 2001). The National Occupational Hazard Survey (conducted from 1972 to 1974) estimated that 58,000 workers in over 30 industries potentially were exposed to isoprene (NIOSH 1976). The National Occupational Exposure Survey (conducted from 1981 to 1983) estimated in a more limited survey that 3,700 workers in four industries, including 578 women, potentially were exposed to isoprene (NIOSH 1990).

## Regulations

***Coast Guard (Dept. of Homeland Security)***

Minimum requirements have been established for safe transport of isoprene on ships and barges.

***Department of Transportation (DOT)***

Isoprene is considered a hazardous material and a marine pollutant, and special requirements have been set for marking, labeling, and transporting this material.

***Environmental Protection Agency (EPA)***

*Clean Air Act*

*New Source Performance Standards:* Manufacture of isoprene is subject to certain provisions for the control of volatile organic compound emissions.

*Prevention of Accidental Release:* Threshold quantity (TQ) = 10,000 lb.

*Clean Water Act*

Isoprene has been designated a hazardous substance.

*Comprehensive Environmental Response, Compensation, and Liability Act*

Reportable quantity (RQ) = 100 lb.

*Emergency Planning and Community Right-To-Know Act*

*Toxics Release Inventory:* Listed substance subject to reporting requirements.

## References


Adam T, Mitschke S, Streibel T, Baker RR, Zimmermann R. 2006. Quantitative puff-by-puff-resolved characterization of selected toxic compounds in cigarette mainstream smoke. *Chem Res Toxicol* 19(4): 511-520.

Akron. 2009. *The Chemical Database*. The Department of Chemistry at the University of Akron. http://ull.chemistry.uakron.edu/erd and search on CAS number. Last accessed: 7/7/09.

Altschuller A. 1983. Natural volatile organic substances and their effect on air quality in the United States. *Atmos Environ* 17(11): 2131-2165.

Barrefors G, Petersson G. 1993. Assessment of ambient volatile hydrocarbons from tobacco smoke and from vehicle emissions. *J Chromatogr* 643(1-2): 71-76.

Borbon A, Fontaine H, Veillerot M, Locoge N, Galloo JC, Guillermo R. 2001. An investigation into the traffic-related fraction of isoprene at an urban location. *Atmos Environ* 35(22): 3749-3760.

Borbon A, Locoge N, Veillerot M, Galloo JC, Guillermo R. 2002. Characterisation of NMHCs in a French urban atmosphere: overview of the main sources. *Sci Total Environ* 292(3): 177-191.

Borbon A, Coddeville P, Locoge N, Galloo JC. 2004. Characterising sources and sinks of rural VOC in eastern France. *Chemosphere* 57(8): 931-942.

Cailleux A, Cogny M, Allain P. 1992. Blood isoprene concentrations in humans and in some animal species. *Biochem Med Metab Biol* 47(2): 157-160.

ChemSources. 2009. *Chem Sources - Chemical Search*. Chemical Sources International. http://www.chemsources.com/chemonline.html and search on isoprene. Last accessed: 7/7/09.

Chiang HL, Tsai JH, Chen SY, Lin KH, Ma SY. 2007. VOC concentration profiles in an ozone non-attainment area: A case study in an urban and industrial complex metroplex in southern Taiwan. *Atmos Environ* 41(9): 1848-1860.

EPA. 2004. *Non-confidential IUR Production Volume Information*. U.S. Environmental Protection Agency. http://www.epa.gov/oppt/iur/tools/data/2002-vol.html and search on CAS number.

Fabiani R, Rosignoli P, De Bartolomeo A, Fuccelli R, Morozzi G. 2007. DNA-damaging ability of isoprene and isoprene mono-epoxide (EPOX I) in human cells evaluated with the comet assay. *Mutat Res* 629(1): 7-13.

Fuentes JD, Wang D. 1999. On the seasonality of isoprene emissions from a mixed temperate forest. *Ecol Appl* 9(4): 1118-1131.

Gelmont D, Stein RA, Mead JF. 1981. Isoprene—the main hydrocarbon in human breath. *Biochem Biophys Res Commun* 99(4): 1456-1460.

Gervasi PG, Citti L, Del Monte M. 1985. Mutagenicity and chemical reactivity of epoxidic intermediates of the isoprene metabolism and other structurally related compounds. *Mutat Res* 156(1-2): 77-82.

Guenther A, Zimmerman P, Wildermuth M. 1994. Natural volatile organic-compound emission rate estimates for U.S. woodland landscapes. *Atmos Environ* 28(6): 1197-1210.

Guenther A, Hewitt CN, Erickson D, Fall R, Geron C, Graedel T, *et al.* 1995. A global model of natural volatile organic compound emissions. *J Geophys Res—Atmos* 100(D5): 8873-8892.

Guo H, Lee SC, Louie PK, Ho KF. 2004. Characterization of hydrocarbons, halocarbons and carbonyls in the atmosphere of Hong Kong. *Chemosphere* 57(10): 1363-1372.

Hagerman LM, Aneja VP, Lonneman WA. 1997. Characterization of non-methane hydrocarbons in the rural southeast United States. *Atmos Environ* 31(23): 4017-4038.

Heavner DL. 1996. Determination of volatile organic compounds and respirable suspended particulate matter in New Jersey and Pennsylvania homes and workplaces. *Environ Int* 22(2): 159-183.

Hellen H, Hakola H, Pirjola L, Laurila T, Pystynen KH. 2006. Ambient air concentrations, source profiles, and source apportionment of 71 different $C_2$-$C_{10}$ volatile organic compounds in urban and residential areas of Finland. *Environ Sci Technol* 40(1): 103-108.

Hong HL, Devereux TR, Melnick RL, Eldridge SR, Greenwell A, Haseman J, Boorman GA, Sills RC. 1997. Both K-*ras* and H-*ras* protooncogene mutations are associated with Harderian gland tumorigenesis in B6C3$F_1$ mice exposed to isoprene for 26 weeks. *Carcinogenesis* 18(4): 783-789.

HSDB. 2009. *Hazardous Substances Data Bank*. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB and search on CAS number. Last accessed: 7/7/09.

IARC. 1994. Isoprene. In *Some Industrial Chemicals*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 60. Lyon, France: International Agency for Research on Cancer. pp. 215-232.

Kuster WC, Jobson BT, Karl T, Riemer D, Apel E, Goldan PD, Fehsenfeld FC. 2004. Intercomparison of volatile organic carbon measurement techniques and data at La Porte during the TexAQS2000 Air Quality Study. *Environ Sci Technol* 38(1): 221-228.

Lamb B, Gay D, Westberg H, Pierce T. 1993. A biogenic hydrocarbon emission inventory for the U.S.A. using a simple forest canopy model. *Atmos Environ Part A—Gen Top* 27(11): 1673-1690.

Leber AP. 2001. Overview of isoprene monomer and polyisoprene production processes. *Chem Biol Interact* 135-136: 169-173.

Lechner M, Moser B, Niederseer D, Karlseder A, Holzknecht B, Fuchs M, Colvin S, Tilg H, Rieder J. 2006. Gender and age specific differences in exhaled isoprene levels. *Respir Physiol Neurobiol* 154(3): 478-483.

Lynch J. 2001. Occupational exposure to butadiene, isoprene and chloroprene. *Chem Biol Interact* 135-136: 207-214.

Mayrhofer S, Heizmann U, Magel E, Eiblmeier M, Müller A, Rennenberg H, Hampp R, Schnitzler JP, Kreuzwieser J. 2004. Carbon balance in leaves of young poplar trees. *Plant Biol* 6(6): 730-739.

Melnick RL, Sills RC. 2001. Comparative carcinogenicity of 1,3-butadiene, isoprene, and chloroprene in rats and mice. *Chem Biol Interact* 135-136: 27-42.

Moukhtar S, Couret C, Rouil L, Simon V. 2006. Biogenic volatile organic compounds (BVOCs) emissions from *Abies alba* in a French forest. *Sci Total Environ* 354(2-3): 232-245.

Mukerjee S. 1997. An environmental scoping study in the lower Rio Grande Valley of Texas—III. Residential microenvironmental monitoring for air, house dust, and soil. *Environ Int* 23(5): 657-673.

NIOSH. 1976. *National Occupational Hazard Survey (1972-74)*. DHEW (NIOSH) Publication No. 78-114. Cincinnati, OH: National Institute for Occupational Safety and Health.

NIOSH. 1990. *National Occupational Exposure Survey (1981-83)*. National Institute for Occupational Safety and Health. Last updated: 7/1/90. http://www.cdc.gov/noes/noes1/40940sic.html.

NTP. 1995. *NTP Technical Report on the Toxicity Studies of Isoprene (CAS No. 78-79-5) Administered by Inhalation to F344/N Rats and B6C3F$_1$ Mice.* NTP Technical Report Series no. 31. Research Triangle Park, NC: National Toxicology Program. pp. 1-G5.

NTP. 1999a. *NTP Report on Carcinogens Background Document for Isoprene*. National Toxicology Program. http://ntp.niehs.nih.gov/files/Isoprene.pdf.

NTP. 1999b. *NTP Toxicology and Carcinogenesis Studies of Isoprene (CAS No. 78-79-5) in F344/N Rats (Inhalation Studies)*. NTP Technical Report Series no. 486. Research Triangle Park, NC: National Toxicology Program. 178 pp.

Parra R, Gasso S, Baldasano JM. 2004. Estimating the biogenic emissions of non-methane volatile organic compounds from the North Western Mediterranean vegetation of Catalonia, Spain. *Sci Total Environ* 329(1-3): 241-259.

Pegoraro E, Rey A, Barron-Gafford G, Monson R, Malhi Y, Murthy R. 2005. The interacting effects of elevated atmospheric $CO_2$ concentration, drought and leaf-to-air vapour pressure deficit on ecosystem isoprene fluxes. *Oecologia* 146(1): 120-129.

Peter H, Wiegand HJ, Bolt HM. 1987. Pharmacokinetics of isoprene in mice and rats. *Toxicol Lett* 36(1): 9-14.

Placke ME, Griffis L, Bird M, Bus J, Persing RL, Cox LA Jr. 1996. Chronic inhalation oncogenicity study of isoprene in B6C3$F_1$ mice. *Toxicology* 113(1-3): 253-262.

Rieder J, Lirk P, Ebenbichler C, Gruber G, Prazeller P, Lindinger W, Amann A. 2001. Analysis of volatile organic compounds: possible applications in metabolic disorders and cancer screening. *Wien Klin Wochenschr* 113(5-6): 181-185.

Sasaki M, Nakamura Y, Fujita K, Kinugawa Y, Iida T, Urahama Y. 2005. Relation between phase structure and peel adhesion of poly(styrene-isoprene-styrene) triblock copolymer/tackifier blend system. *J Adhes Sci Technol* 19(16): 1445-1457.

Schnitzler JP, Graus M, Kreuzwieser J, Heizmann U, Rennenberg H, Wisthaler A, Hansel A. 2004. Contribution of different carbon sources to isoprene biosynthesis in poplar leaves. *Plant Physiol* 135(1): 152-160.

Schnitzler JP, Zimmer I, Bachl A, Arend M, Fromm J, Fischbach RJ. 2005. Biochemical properties of isoprene synthase in poplar (*Populus* x *canescens*). *Planta* 222(5): 777-786.

Senthilmohan ST, Milligan DB, McEwan MJ, Freeman CG, Wilson PF. 2000. Quantitative analysis of trace gases of breath during exercise using the new SIFT-MS technique. *Redox Rep* 5(2-3): 151-153.

Senthilmohan ST, McEwan MJ, Wilson PF, Milligan DB, Freeman CG. 2001. Real time analysis of breath volatiles using SIFT-MS in cigarette smoking. *Redox Rep* 6(3): 185-187.

Sharkey TD. 2005. Effects of moderate heat stress on photosynthesis: Importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant Cell Environ* 28(3): 269-277.

Simon V, Dumergues L, Ponche JL, Torres L. 2006. The biogenic volatile organic compounds emission inventory in France: application to plant ecosystems in the Berre-Marseilles area (France). *Sci Total Environ* 372(1): 164-182.

So KL, Wang T. 2004. $C_3$–$C_{12}$ non-methane hydrocarbons in subtropical Hong Kong: spatial-temporal variations, source-receptor relationships and photochemical reactivity. *Sci Total Environ* 328(1-3): 161-174.

SRI. 2009. *Directory of Chemical Producers*. Menlo Park, CA: SRI Consulting. Database edition. Last accessed: 7/13/09.

Taalman R. 1996. Isoprene: Background and issues. *Toxicology* 113(1-3): 242-246.

Tambunan P, Baba S, Kuniyoshi A, Iwasaki H, Nakamura T, Yamasaki H, Oku H. 2006. Isoprene emission from tropical trees in Okinawa Island, Japan. *Chemosphere* 65(11): 2138-2144.

Taucher J, Hansel A, Jordan A, Fall R, Futrell JH, Lindinger W. 1997. Detection of isoprene in expired air from human subjects using proton-transfer-reaction mass spectrometry. *Rapid Commun Mass Spectrom* 11(11): 1230-1234.

Tice RR. 1988. The cytogenetic evaluation of *in vivo* genotoxic and cytotoxic activity using rodent somatic cells. *Cell Biol Toxicol* 4(4): 475-486.

Tice RR, Boucher R, Luke CA, Paquette DE, Melnick RL, Shelby MD. 1988. Chloroprene and isoprene: cytogenetic studies in mice. *Mutagenesis* 3(2): 141-146.

Turner C, Spanel P, Smith D. 2006. A longitudinal study of breath isoprene in healthy volunteers using selected ion flow tube mass spectrometry (SIFT-MS). *Physiol Meas* 27(1): 13-22.

USITC. 2009. *USITC Interactive Tariff and Trade DataWeb.* United States International Trade Commission. http://dataweb.usitc.gov/scripts/user_set.asp and search on HTS no. 2901242000. Last accessed: 7/7/09.

Warneke C, Kato S, De Gouw JA, Goldan PD, Kuster WC, Shao M, Lovejoy ER, Fall R, Fehsenfeld FC. 2005. Online volatile organic compound measurements using a newly developed proton-transfer ion-trap mass spectrometry instrument during New England Air Quality Study—Intercontinental Transport and Chemical Transformation 2004: performance, intercomparison, and compound identification. *Environ Sci Technol* 39(14): 5390-5397.

Zhao WX, Hopke PK, Karl T. 2004. Source identification of volatile organic compounds in Houston, Texas. *Environ Sci Technol* 38(5): 1338-1347.