

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

|   |   |
|---|---|
| <b>MAK value (2008)</b>   | <b>3 ml/m<sup>3</sup> <math>\triangleq</math> 8.4 mg/m<sup>3</sup></b>                                    |
| <b>Peak limitation (2008)</b>   | <b>Peak Limitation Category II, excursion factor 8</b>  |
| <b>Absorption through the skin</b>  | –   |
| <b>Sensitization</b>  | –   |
| <b>Carcinogenicity (2008)</b>   | <b>Category 5</b>   |
| <b>Prenatal toxicity (2008)</b>   | <b>Pregnancy Risk Group C</b>   |
| <b>Germ cell mutagenicity (2008)</b>  | <b>Category 5</b>   |
| <b>BAT value</b>  | –   |
| <b>Synonyms</b>   | Hemiterpene<br>Isopentadiene<br><br>$\beta$ -methylbivinyI<br>2-methylbutadiene<br>2-methylbuta-1,3-diene |
| <b>Chemical name</b>  | 2-methylbuta-1,3-diene  |
| <b>CAS number</b>   | 78-79-5   |
| <b>Formula</b>  | H <sub>2</sub> C= C(CH <sub>3</sub> )–CH=CH <sub>2</sub><br>C <sub>5</sub> H <sub>8</sub>                 |
| <b>Molecular weight</b>   | 68.12 g/mol   |
| <b>Melting point</b>  | –120°C (CambridgeSoft 2006)   |
| <b>Boiling point</b>  | 34°C (CambridgeSoft 2006)   |
| <b>Density at 20°C</b>  | 0.681–0.69 g/cm <sup>3</sup> (BG Chemie 2000)   |
| <b>Vapour pressure at 25°C</b>  | 733.3 hPa (SRC 2006)  |
| <b>log K<sub>ow</sub></b>   | 2.42 (SRC 2006)   |
| <b>Solubility</b>   | 642 mg/l water (SRC 2006)   |
| <b>1 ml/m<sup>3</sup> (ppm) <math>\triangleq</math> 2.79 mg/m<sup>3</sup></b> | <b>1 mg/m<sup>3</sup> <math>\triangleq</math> 0.358 ml/m<sup>3</sup> (ppm)</b>                            |

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 (copolymerised 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<sup>3</sup> were found in 398 of 435 samples (91.3%), of 1–5 ml/m<sup>3</sup> in 25 samples (5.8%), 5–10 ml/m<sup>3</sup> in 6 samples (1.4%), and more than 10 ml/m<sup>3</sup> (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<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup> and above in mice. In the males, degeneration of the olfactory epithelium was observed at 7000 ml/m<sup>3</sup>. However, no substance-specific effects occurred in rats on exposure up to 7000 ml/m<sup>3</sup>. 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<sup>3</sup> 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<sup>3</sup>. In rats, isoprene concentrations of up to 7000 ml/m<sup>3</sup> had no effects on fertility. In mice, developmental toxicity such as reduced body weight of foetuses was observed after inhalation at 1400 ml/m<sup>3</sup> and above. Maternal toxicity occurred at 7000 ml/m<sup>3</sup>. In rats, isoprene produces neither developmental nor maternal toxicity after inhalation up to 7000 ml/m<sup>3</sup>.

## 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<sub>N</sub>2 type reaction at the C2 atom, which occurs with the butadiene monoepoxides, is suppressed by the methyl group. Preference is given to the S<sub>N</sub>1 type reaction, for which reason the monoepoxides of isoprene are rapidly hydrolysed. An S<sub>N</sub>2 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-diepoxibutane, 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<sup>3</sup>. 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<sup>3</sup> 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 <sup>a)</sup> ± standard deviation) (range) | Converted concentration in pulmonary exhaled air (mean value <sup>a)</sup> ± standard deviation) (range) [ $\mu\text{l}/\text{m}^3$ ] | References                                    |
|----------------------|---------------------|---|---|---|
| 13                   | – <sup>9)</sup>     | 230 (90–450) [ $\mu\text{l}/\text{m}^3$ ]   | 230   | Jansson and Larsson 1969                      |
| 25                   | 11 ♀, 14 ♂ (adults) | 28.4 ± 12.3 [nmol/l]<br>28.3 ± 7.4 [nmol/l]   | 477 ± 206 <sup>1)</sup><br>475 ± 124 <sup>1)</sup>  | DeMaster and Nagasawa 1978                    |
| 54                   | 19 ♀, 35 ♂ (18–60)  | 28.9 <sup>4)</sup> [ng/l]   | 11 <sup>1), 4)</sup>  | Krotoszynski et al. 1979                      |
| 50                   | 30 ♀, 20 ♂ (15–60)  | 14.6 ± 6.4 [nmol/l]   | 367 ± 161 <sup>1)</sup>   | Cailleux and Alain 1989                       |
| 5                    | ♂                   | 0.99 ± 0.58 [nmol/l]  | 25 ± 15 <sup>1)</sup>   | Phillips and Greenberg 1991                   |
| 12                   | (20–30)             | 1.62 ± 0.976 <sup>6)</sup> [nmol/l]   | 41 ± 25 <sup>1)</sup>   | Kohlmüller and Kochen 1993                    |
| 5                    | 5 ♂ (18–50)         | 21.7 ± 6.4 [nmol/l]   | 364 ± 107 <sup>1)</sup>   | Stone et al. 1993                             |
| 43                   | 23 ♀, 20 ♂ (22–75)  | 7.05 ± 3.53 [nmol/l]  | 118 ± 59 <sup>1)</sup>  | Mendis et al. 1994                            |
| 15                   | – <sup>9)</sup>     | 7.1 ± 1.0 [nmol/l]  | 119 ± 17 <sup>1)</sup>  | Mendis et al. 1995                            |
| ≥ 40                 | – <sup>9)</sup>     | 250 (70–580) [ $\mu\text{l}/\text{m}^3$ ]   | – <sup>3)</sup>   | Hansel et al. 1995                            |
| 16                   | 10 ♀, 6 ♂ (adults)  | 3.89 ± 2.43 [nmol/l]<br>3.46 ± 0.84 [nmol/l]  | 98 ± 61 <sup>1)</sup><br>87 ± 21 <sup>1)</sup>  | Jones et al. 1995                             |
| 10                   | 9♂, 1 ♀ (adults)    | (0.37–3.2) [nmol/l]   | (9–80) <sup>1)</sup>  | Foster et al. 1996                            |
| 4                    | 1 ♀, 3♂ (19–34)     | 4.7 [nmol/l]  | 117 ± 11 <sup>1), 5)</sup>  | Filser et al. 1996; Csanády and Filser 2001 b |
| 141                  | (22–74)             | 240 ± 120 [ $\mu\text{l}/\text{m}^3$ ]  | – <sup>3)</sup>   | Taucher et al. 1997                           |

**Table 1** (Continued)

| Number of volunteers | Sex (age in years)               | Concentration measured in exhaled air (mean value <sup>a)</sup> ± standard deviation) (range) |                              | Converted concentration in pulmonary exhaled air (mean value <sup>a)</sup> ± standard deviation) (range) [ $\mu\text{l}/\text{m}^3$ ] | References               |
|----------------------|----------------------------------|---|------------------------------|---|--------------------------|
| 1                    | — <sup>9)</sup>                  | 10 ± 1.4  | [nmol/l]                     | 168 ± 23 <sup>1)</sup>  | Grote and Pawliszyn 1997 |
| 10                   | — <sup>9)</sup>                  | (30–135)  | [ $\mu\text{l}/\text{m}^3$ ] | — <sup>3)</sup>   | Fenske and Paulson 1999  |
| 6                    | 1 ♀, 5 ♂ (24–60)                 | 95, 58, 54, 113, 92, 48   | [ $\mu\text{l}/\text{m}^3$ ] | 51 ± 18 <sup>2)</sup> , <sup>6)</sup>   | Smith et al. 1999        |
| 29                   | ♀ and ♂ (25–65)                  | 83 ± 45   | [ $\mu\text{l}/\text{m}^3$ ] | 55 ± 30 <sup>2)</sup>   | Španělet al. 1999        |
| 1                    | — <sup>9)</sup>                  | 3 (2–4)   | [nmol/l]                     | 75 <sup>1)</sup>  | Hyšpler et al. 2000      |
| 10                   | 2 ♀, 8 ♂ (22–59)                 | 1.7 (0.5–2.5)   | [nmol/l]                     | 43 <sup>1)</sup>  | Mitsui et al. 2000       |
| 8                    | 3 ♀, 5 ♂ (15–30)                 | (55–185)  | [ $\mu\text{l}/\text{m}^3$ ] | (55–185)  | Senthilmohan et al. 2000 |
| 17                   | 9 ♀, 8 ♂ (24–62)                 | 89 ± 36   | [ $\mu\text{l}/\text{m}^3$ ] | 59 ± 24 <sup>2)</sup>   | Davies et al. 2001       |
| 8                    | (adults, at rest)                | 157 ± 67  | [ $\mu\text{l}/\text{m}^3$ ] | — <sup>3)</sup>   | Karl et al. 2001         |
| 8                    | (adults, physically active)      | 385 ± 140   | [ $\mu\text{l}/\text{m}^3$ ] | — <sup>3)</sup>   | Karl et al. 2001         |
| 31                   | 10 ♀, 21 ♂ (30–46)               | 1.12 ± 0.14 <sup>7)</sup>   | [nmol/l]                     | 28 ± 20 <sup>1)</sup> , <sup>6)</sup>   | McGrath et al. 2001      |
| 4                    | 2 ♀, 2 ♂ (28–39)                 | 2.4 ± 0.90  | [nmol/l]                     | 40 ± 15 <sup>1)</sup>   | Lärstad et al. 2002      |
| 10                   | 4 ♀, 6 ♂ (28–41)                 | 5.99  | [nmol/l]                     | 151 <sup>1)</sup>   | Scholpp et al. 2002      |
| 5                    | 2 ♀, 3 ♂ (27–65)                 | 82 ± 42 <sup>6)</sup> (55–121)  | [ $\mu\text{l}/\text{m}^3$ ] | — <sup>3)</sup>   | Diskin et al. 2003       |
| 16                   | 8 ♀, 8 ♂ (adults)                | 6.07 ± 1.75   | [nmol/l]                     | 102 ± 29 <sup>1)</sup>  | Cope et al. 2004         |
| 50                   | 23 ♀, 27 ♂ (55.7 <sup>8)</sup> ) | 3.8 ± 3.9 <sup>6)</sup>   | [nmol/l]                     | 64 ± 65 <sup>1)</sup>   | Poli et al. 2005         |

**Table 1** (Continued)

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

<sup>a)</sup> unless otherwise stated;

<sup>1)</sup> conversion of nmol/l to  $\mu\text{l}/\text{m}^3$  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  $\mu\text{l}/\text{m}^3$  by multiplication with 0.369;

<sup>2)</sup> conversion of alveolar to pulmonary concentration by multiplication with  $2/3$  (according to Fiserova-Bergerova 1983);

<sup>3)</sup> not determinable from the method described, whether alveolar or pulmonary exhaled air;

<sup>4)</sup> geometric mean value;

<sup>5)</sup> standard deviation given by authors;

<sup>6)</sup> standard deviation calculated from given values or parameters;

<sup>7)</sup> standard error;

<sup>8)</sup> median;

<sup>9)</sup> 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 waking 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  $\mu\text{l}/\text{m}^3$ . In one volunteer, the mean isoprene concentration was only 5  $\mu\text{l}/\text{m}^3$  (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 hypercholesterolemia 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  $37 \pm 25$  nmol/l (mean value  $\pm$  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  $10.29 \pm 6.17$  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  $6.68 \pm 4.71$  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  $5.2 \pm 4.0$  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)  $^{14}\text{C}$ -isoprene concentrations of 0, 8, 260, 1480 or 8200 ml/m<sup>3</sup> up to six hours. The  $^{14}\text{C}$  levels in urine and faeces, the exhaled substances and the  $^{14}\text{C}$  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  $^{14}\text{C}$ -isoprene and the retention of  $^{14}\text{C}$ -isoprene were estimated through comparison with the  $^{14}\text{C}$  still present in the organism at the end of exposure. This was 19% at 8 ml/m<sup>3</sup>, 9.1% at 260 ml/m<sup>3</sup>, 5.8% at 1480 ml/m<sup>3</sup> and 4.5% at 8200 ml/m<sup>3</sup>. In the same way, the metabolized portion of the inhaled  $^{14}\text{C}$ -isoprene was estimated by comparing the calculated quantities of inhaled  $^{14}\text{C}$ -isoprene with the  $^{14}\text{C}$  quantities retrieved in urine, faeces, total organism and exhaled  $^{14}\text{CO}_2$  up to 66 hours after end of exposure. This was 25.3% at 8 ml/m<sup>3</sup>, 12.0% at 260 ml/m<sup>3</sup>, 4.7% at 1480 ml/m<sup>3</sup>

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

Male B6C3F<sub>1</sub> mice were exposed (nose-only) by inhalation to <sup>14</sup>C-labelled or non-labelled isoprene at concentrations of 0, 20, 200 or 2000 ml/m<sup>3</sup> 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<sup>3</sup>), 830 ng/ml (200 ml/m<sup>3</sup>) or 6800 ng/ml (2000 ml/m<sup>3</sup>). The <sup>14</sup>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<sup>3</sup>, 8.9% at 205 ml/m<sup>3</sup> and 3.8% at 2000 ml/m<sup>3</sup>, related to the inhaled quantity of <sup>14</sup>C-isoprene. Between 52% (at 18 ml <sup>14</sup>C-isoprene/m<sup>3</sup>) and 73% (at 2000 ml <sup>14</sup>C-isoprene/m<sup>3</sup>) of the metabolite-associated radioactivity was excreted in the urine over a 64-hour post-exposure period. The amounts of inhaled <sup>14</sup>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<sup>3</sup> they amounted to 4.6%, 7.5% and 2.3% (Bond et al. 1991).

Groups of three male F344 rats and four male B6C3F<sub>1</sub> mice received single intraperitoneal injections of <sup>14</sup>C-isoprene in doses of 64 mg/kg body weight in corn oil. Of this, about 50% unchanged <sup>14</sup>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-butenic 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/l and a log K<sub>ow</sub> of 2.42, dermal fluxes of 0.023 or 0.026 mg/cm<sup>2</sup> 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<sup>2</sup>) 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).



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<sub>1</sub> 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

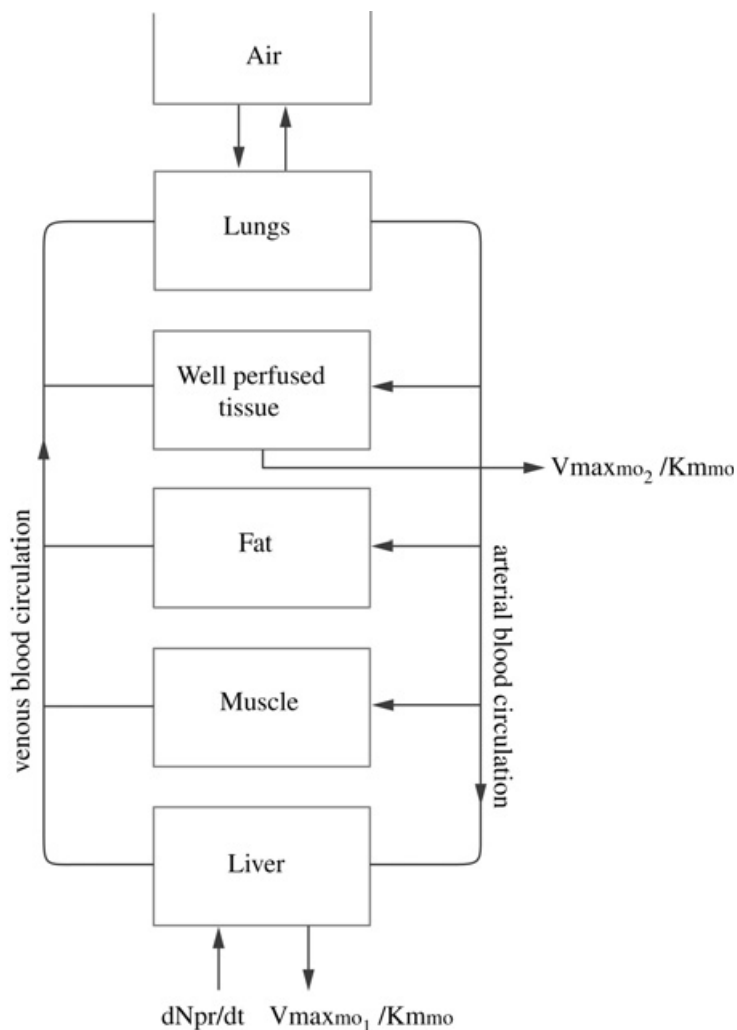
Groups of three male Sprague Dawley rats and three male B6C3F<sub>1</sub> mice were given intraperitoneal injections of <sup>14</sup>C-isoprene in single doses of 0.3, 3, 300, 1000 or 3000 µmol/kg body weight (about 0.02, 0.2, 20.4, 68.1 or 204.4 mg/kg body weight) or daily doses of 500 µmol/kg body 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. <sup>14</sup>C adduct at  $0.158 \pm 0.035$  pmol/mg globin was formed by mice and  $0.079 \pm 0.016$  pmol/mg globin by rats in relation to the retained isoprene/kg body weight. Repeated administration of <sup>14</sup>C-isoprene at 34 mg/kg body weight produced an accumulation of <sup>14</sup>C-haemoglobin adducts (Sun et al. 1989).

Groups of four male B6C3F<sub>1</sub> mice were exposed for 6 hours to <sup>14</sup>C-isoprene concentrations of 20, 200 or 2000 ml/m<sup>3</sup>. Twenty-four hours thereafter, the corresponding haemoglobin adduct levels were  $11 \pm 0.5$ ,  $90 \pm 13$  and  $170 \pm 13$  pmol/mg globin. In the range of 200 to 2000 ml/m<sup>3</sup>, 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:  $dN_{pr}/dt$ , endogenous formation rate;

$V_{maxmo1}$ ,  $V_{maxmo2}$ , maximum metabolic rate in liver or well perfused tissue;

$K_{mmo}$ , 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<sup>3</sup> and not until beyond this value in the mouse.

The half-maximum metabolic rate was reached at 360 ml/m<sup>3</sup> in the mouse and at 180 ml/m<sup>3</sup> in the rat. In the low concentration range, for which the model predictions in volunteers were experimentally checked at isoprene concentrations ≤ 50 ml/m<sup>3</sup>, 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<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup>, 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  $\mu\text{mol/h}$  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  $5.2 \pm 4.0$  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 B6C3F<sub>1</sub> 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



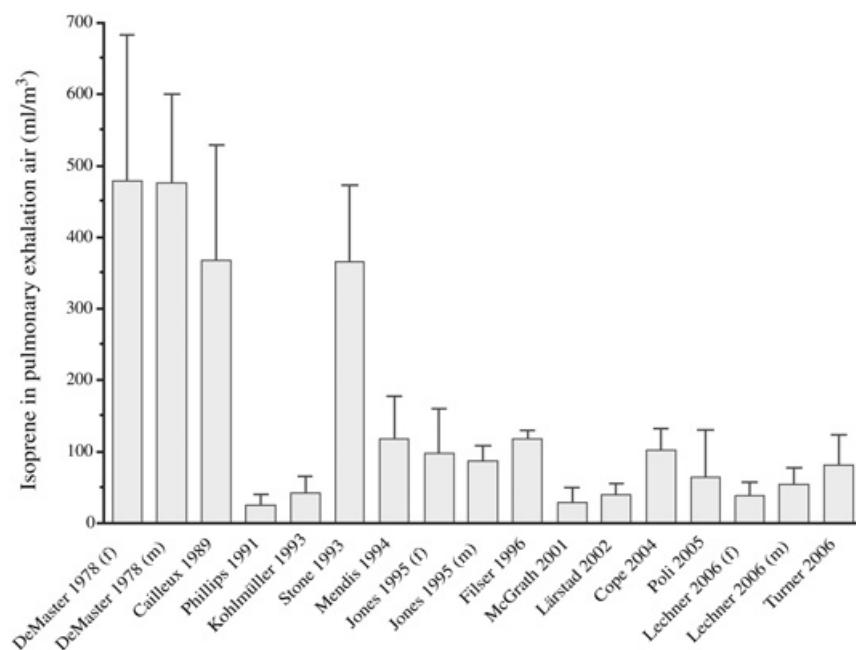
an additional AUC equal to the AUC from the standard deviation of endogenous isoprene concentration. The MAK value for ethanol was already derived according to the same principle. Ethanol is also formed endogenously and transformed into carcinogenic metabolites.

### 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  $\pm$  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  $0.064 \text{ ml/m}^3$  and a weighted standard deviation of  $0.049 \text{ ml/m}^3$  after Sachs (1997) was calculated for the 337 volunteers considered.

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

Endogenous isoprene:

|                               |  |
|-------------------------------|--|
| Rate of formation             | $13.1 \pm 10.0 \text{ } \mu\text{mol/h}$     |
| Concentration in venous blood | $5.2 \pm 4.0 \text{ nmol/l}$                 |
| AUC (0–80 years)              | $3.6 \pm 2.8 \text{ mmol} \times \text{h/l}$ |

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

|  |                                      |
|--|--------------------------------------|
| Additional AUC (40 years)                            | $9.8 \text{ mmol} \times \text{h/l}$ |
| Expected concentration in venous blood after 8 hours | $133 \text{ nmol/l}$                 |

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  $2.9 \text{ ml/m}^3$  in the ambient air is obtained.

The AUC after an 8-hour daily exposure to about  $3 \text{ ml/m}^3$  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  $278\text{--}27\,800 \text{ mg/m}^3$  (about  $100\text{--}10\,000 \text{ ml/m}^3$ ) for 5 minutes. Isoprene concentrations of  $278 \text{ mg/m}^3$  (about  $100 \text{ ml/m}^3$ ) 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/m <sup>3</sup> (about 100–10 000 ml/m <sup>3</sup> ); no further details | no data on analytics | 278 mg/m <sup>3</sup> (about 100 ml/m <sup>3</sup> ): odour perceptible; 13 900 mg/m <sup>3</sup> (about 5000 ml/m <sup>3</sup> ): headache; 27 800 mg/m <sup>3</sup> (10 000 ml/m <sup>3</sup> ): marked bronchial irritation (no further details) | BG Chemie 2000  |
| 10 volunteers; no further details | no data on duration; inhalation; 160 mg/m <sup>3</sup> (about 57 ml/m <sup>3</sup> ); no further details  | no data on analytics | mild irritation of mucous membranes in nose, larynx and pharynx; odour threshold: 10 mg/m <sup>3</sup> (about 3.6 ml/m <sup>3</sup> ) (no further details)  | Gostinskii 1965 |

limit of odour perception, 695 mg/m<sup>3</sup> (about 250 ml/m<sup>3</sup>) clearly perceptible and 2780 mg/m<sup>3</sup> (about 1000 ml/m<sup>3</sup>) very perceptible. In addition, headache and pronounced headache occurred at 13 900 mg/m<sup>3</sup> (about 5000 ml/m<sup>3</sup>) and 27 800 mg/m<sup>3</sup> (about 10 000 ml/m<sup>3</sup>), 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/m<sup>3</sup> (about 57 ml/m<sup>3</sup>) by 10 volunteers produced mild mucosal irritation in nose, larynx and pharynx. The odour threshold was cited as being 10 mg/m<sup>3</sup> (about 3.6 ml/m<sup>3</sup>) (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                               |
|---|---|---|--|
| <b>rat</b> ,<br>Wistar,<br>20 ♂/♀<br>per group        | ♂: 27 600–100 900 mg/m <sup>3</sup><br>(9893–36 165 ml/m <sup>3</sup> );<br>4 h<br>51 500 mg/m <sup>3</sup><br>(18 459 ml/m <sup>3</sup> ); 1 h<br>♀: 29 500–98 100 mg/m <sup>3</sup><br>(10 574–35 161 ml/m <sup>3</sup> );<br>4 h | 18 459 ml/m <sup>3</sup> (1 h): LC <sub>50</sub> not reached;<br>36 165/35 161 ml/m <sup>3</sup> (4 h): LC <sub>50</sub> not reached;<br>transiently poor general condition at the highest concentration  | BG Chemie<br>2000                        |
| <b>rat</b> ,<br>no further details                    | no further details; 4 h   | 180 000 mg/m <sup>3</sup> (64 516 ml/m <sup>3</sup> ): LC <sub>50</sub> ; no further details  | Shugaev<br>1969                          |
| <b>rat</b> ,<br>Wistar,<br>10 ♂ per group             | 0.26; 0.81; 2.18; 4.98; 8.40;<br>21.44 mg/l<br>(260–21 440 ml/m <sup>3</sup> ); 4 h   | at 8400 ml/m <sup>3</sup> and above: thymus after 24 h: cell count and mitotic index decreased, absolute and relative weight decreased; reversible after 3 d  | Mamedov<br>1978                          |
| <b>mouse</b> ,<br>NMRI,<br>10 ♂ per group             | 14 100, 31 500 mg/m <sup>3</sup><br>(about 5054,<br>11 290 ml/m <sup>3</sup> ); 4 h   | 11 290 ml/m <sup>3</sup> : no mortality, no further details   | BG Chemie<br>2000                        |
| <b>mouse</b> ,<br>10 per group,<br>no further details | 50 000–150 000 mg/m <sup>3</sup><br>(17921–53763 ml/m <sup>3</sup> );<br>2 h, recovery period:<br>21 days,<br>no further details  | 53 763 ml/m <sup>3</sup> : LC <sub>50</sub> 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<br>2000                        |
| <b>mouse</b> ,<br>♂, ♀, no further details            | no further details;<br>40 min,<br>2 h   | at 750 mg/m <sup>3</sup> (268 ml/m <sup>3</sup> ) and above (40 min): central nervous effects;<br>139 000 mg/m <sup>3</sup><br>(49 821 ml/m <sup>3</sup> )/148 000 mg/m <sup>3</sup><br>(53 047 ml/m <sup>3</sup> ) (2 h): ♂/♀: LC <sub>50</sub> ,<br>irritating, narcotizing, enlarged lungs and congestion in the lungs | BG Chemie<br>2000;<br>Gostinskii<br>1965 |
| <b>mouse</b> ,<br>no further details                  | no further details; 2 h   | 157 000 mg/m <sup>3</sup> (56 272 ml/m <sup>3</sup> ): LC <sub>50</sub>   | Shugaev<br>1969                          |
| <b>mouse</b> ,<br>no further details                  | 100 000–120 000 mg/m <sup>3</sup><br>(35 843–50 179 ml/m <sup>3</sup> );<br>no further details  | 35 843–50 179 ml/m <sup>3</sup> : deep narcosis;<br>50 179 ml/m <sup>3</sup> : fatal  | BG Chemie<br>2000                        |

**Table 3** (Continued)

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

### 5.1.1 Inhalation

The LC<sub>50</sub> of rats after 4-hour inhalation exposure was about 65 000 ml/m<sup>3</sup> and that of mice after 2-hour inhalation about 50 000 ml/m<sup>3</sup> (BG Chemie 2000). In male rats, reversible effects in the thymus were found 24 hours after 4-hour exposure to 8400 ml/m<sup>3</sup> and above (Mamedov 1978). Decreased respiration rates (about 4%) were found in male mice that had been exposed to 465 ml/m<sup>3</sup> 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<sup>3</sup> was given for mice (Wilkins et al. 2001; Wolkoff et al. 2000). In mice exposed to 20, 200 or 2000 ml/m<sup>3</sup>, 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<sup>3</sup> (Dahl et al. 1987). Rabbits exposed up to 1470 ml/m<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup>, cell proliferation in the thymus was in-

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

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



**Table 4** (Continued)

| Species, strain, no. of animals per sex                      | Duration, concentration   | Findings  | References                                |
|--|---|---|---|
| <b>rat,</b><br>F344/N,<br>10 ♂/♀ per group                   | <b>13 weeks,</b><br>0, 70, 220, 700,<br>2200, 7000 ml/m <sup>3</sup> ,<br>6 h/day, 5 days/wk,<br>purity: > 99%  | <b>7000 ml/m<sup>3</sup>:</b> 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             |
| <b>rat,</b><br>F344/N,<br>40 ♂ per group                     | <b>6 months,</b><br>0, 70, 220, 700,<br>2200, 7000 ml/m <sup>3</sup> ,<br>6 h/day, 5 days/wk,<br>per group 10 animals investigated;<br>recovery period 6 months<br>(per group 30 ♂),<br>purity: > 99% | <b>after 6 months:</b><br><b>7000 ml/m<sup>3</sup>:</b> Leydig cell hyperplasia (10/10, control: 1/10);<br><b>after 12 months (6 months exposure and 6 months recovery period): at 70 ml/m<sup>3</sup> and above:</b> Leydig cell hyperplasia in all exposed groups: 30/30, control: 25/30  | Melnick et al. 1992, 1994, 1996; NTP 1995 |
| <b>rat,</b><br>F344/N,<br>50 ♂/♀ per group                   | <b>105 weeks,</b><br>0, 220, 700,<br>7000 ml/m <sup>3</sup> ,<br>6 h/day, 5 days/wk   | <b>at 700 ml/m<sup>3</sup> and above:</b> ♂: fibrotic changes in the spleen, renal tubular hyperplasia;<br><b>7000 ml/m<sup>3</sup>:</b> ♂: hyperplasia in the parathyroid gland, ♀: purulent inflammation in the nose, bile duct hyperplasia<br>(compare Section 5.7.2)  | NTP 1999                                  |
| <b>mouse,</b><br>B6C3F <sub>1</sub> ,<br>per group<br>10 ♂/♀ | <b>2 weeks,</b><br>0, 438, 875, 1750,<br>3500, 7000 ml/m <sup>3</sup> ,<br>6 h/day, 5 days/wk,<br>purity: > 99%   | <b>at 438 ml/m<sup>3</sup> and above:</b> ♂, ♀: haematocrit decreased, haemoglobin decreased, erythrocyte count decreased, relative liver weight increased, absolute thymus weight decreased, epithelial hyperplasia in the forestomach;<br>♂: body weight decreased, cytoplasmic vacuolization of hepatocytes; absolute and relative spleen weight decreased, absolute testis weight decreased;<br>♀: absolute liver weight increased, relative thymus weight decreased;<br><b>at 875 ml/m<sup>3</sup> and above:</b> ♂: absolute liver weight increased, relative thymus- and testis weight decreased;<br><b>at 1750 ml/m<sup>3</sup> and above:</b> ♂: degeneration of the olfactory epithelium; | Melnick et al. 1990; NTP 1995             |

**Table 4** (Continued)

| Species,<br>strain,<br>no. of<br>animals per<br>sex          | Duration,<br>concentration   | Findings   | References   |
|--|--|--|--|
|  |  | <b>at 3500 ml/m<sup>3</sup> and above:</b> ♀: absolute and relative spleen weight decreased;<br><b>7000 ml/m<sup>3</sup>:</b> ♂: body weight gain decreased, atrophy of the thymus and of the testes   |  |
| <b>mouse,</b><br>no further<br>details                       | <b>20 days,</b><br>60 000 mg/m <sup>3</sup><br>(about 21 503 ml/<br>m <sup>3</sup> ),<br>2 h/day<br>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<br>2000                                  |
| <b>mouse,</b><br>B6C3F <sub>1</sub> ,<br>10 ♂/♀ per<br>group | <b>13 weeks,</b><br>0, 70, 220, 700,<br>2200, 7000 ml/m <sup>3</sup> ,<br>6 h/day, 5 days/<br>wk, purity: > 99%        | <b>70 ml/m<sup>3</sup>:</b> ♀: body weight gain not concentration-dependently decreased;<br><b>220 ml/m<sup>3</sup>:</b> ♂: relative spleen weight decreased, ♀: absolute kidney weight increased;<br><b>at 700 ml/m<sup>3</sup> and above:</b> ♂, ♀: 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><b>at 2200 ml/m<sup>3</sup> and above:</b> ♂: Howell-Jolly bodies increased, cytoplasmic vacuolization in the liver, absolute and relative testis weight decreased;<br><b>7000 ml/m<sup>3</sup>:</b> ♂: 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<br>et al. 1994;<br>NTP 1995                |
| <b>mouse,</b><br>B6C3F <sub>1</sub> ,<br>40 ♂ per<br>group   | <b>6 months,</b><br>0, 70, 220, 700, 2200,<br>7000 ml/m <sup>3</sup> , 6 h/day,<br>5 day/wk, per group                 | <b>after 6 months:</b><br><b>70 ml/m<sup>3</sup>:</b> NOAEC;<br><b>at 220 ml/m<sup>3</sup> and above:</b> grip strength of fore- and hindlimbs decreased;  | Melnick<br>et al. 1992,<br>1994, 1996;<br>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%  | <p><b>at 700 ml/m<sup>3</sup> and above:</b> epithelial hyperplasia of the forestomach, macrocytic anaemia; <b>7000 ml/m<sup>3</sup>:</b> 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</p> <p><b>6 months exposure and 6 months recovery period:</b></p> <p><b>at 70 ml/m<sup>3</sup> and above:</b> degeneration the white matter of spinal cord;</p> <p><b>at 220 ml/m<sup>3</sup> and above<sup>3</sup>:</b> degeneration of the olfactory epithelium in nasal turbinates;</p> <p><b>at 700 ml/m<sup>3</sup> and above:</b> epithelial hyperplasia in the forestomach and the alveoli, hepatocellular foci increased;</p> <p><b>7000 ml/m<sup>3</sup>:</b> mortality increased, tumour incidence increased; compare Section 5.7.2</p> |                        |
| <b>mouse,</b><br>B6C3F <sub>1</sub> ,<br>50 ♂ per group | <b>80 weeks,</b><br>0, 10, 70, 280, 700,<br>2200 ml/m <sup>3</sup> , 8 h/day,<br>5 days/wk,<br>(2200 ml/m <sup>3</sup> ,<br>4 and 8 h/day),<br>purity: ≥ 99% | <p><b>at 10 ml/m<sup>3</sup> and above:</b> proliferation of haematopoietic cells in the spleen, myeloid hyperplasia in the bone marrow;</p> <p><b>at 280 ml/m<sup>3</sup> and above:</b> survival &lt; 50% (no further details), absolute and relative testis weight decreased, mild metaplasia of the olfactory epithelium to respiratory epithelium; tumours compare Section 5.7.2</p>  | Placke et al.<br>1996  |
| <b>mouse,</b><br>B6C3F <sub>1</sub> ,<br>50 ♀ per group | <b>80 weeks,</b><br>0, 10, 70 ml/m <sup>3</sup> ,<br>8 h/day, 5 days/<br>wk, purity: ≥ 99%   | <p><b>at 10 ml/m<sup>3</sup> and above:</b> proliferation of haematopoietic cells in the spleen, myeloid hyperplasia in the bone marrow;</p> <p><b>70 ml/m<sup>3</sup>:</b> slight metaplasia of the olfactory epithelium into respiratory epithelium; for tumours see Section 5.7.2</p>   | Placke et al.<br>1996  |
| <b>rabbit,</b><br>(no further details)                  | <b>4 months,</b><br>0, 400 mg/m <sup>3</sup><br>(143 ml/m <sup>3</sup> ),<br>4 h/day (no further details), room temperature or 30–32°C                       | <p><b>143 ml/m<sup>3</sup>:</b> at room temperature: activity of immune capacities decreased (no further details);</p> <p><b>at 32°C:</b> inhibition of immunological reaction ability (no further details)</p>  | Samedov<br>et al. 1978 |

**Table 4** (Continued)

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

creased in male Wistar rats and cell count and mitotic index in the thymus were decreased at 364.2 ml/m<sup>3</sup> (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/m<sup>3</sup>. 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/m<sup>3</sup> and above was also found in the males of the 7000 ml/m<sup>3</sup> 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/m<sup>3</sup> and at the end of the recovery period in the animals of all concentration groups.

In the rats of the 7000 ml/m<sup>3</sup> 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/m<sup>3</sup> is also the LOAEC.

In an NTP carcinogenicity study, male and female F344/N rats inhaled 0, 220, 700 or 7000 ml/m<sup>3</sup> 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/m<sup>3</sup> 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<sup>3</sup>. 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<sup>3</sup> (about 21 505 ml/m<sup>3</sup>) 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<sub>1</sub> 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<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup> and above. Atrophy of the testes and the thymus was diagnosed at 7000 ml/m<sup>3</sup> (Melnick et al. 1990; NTP 1995). No NOAEC was obtained.

In a further NTP study, B6C3F<sub>1</sub> 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<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup> in the female mice, no NOAEC can be given.

In a further NTP study involving male B6C3F<sub>1</sub> 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<sup>3</sup> and above was found after six months. Epithelial hyperplasia of the forestomach was found at 700 ml/m<sup>3</sup> and above, which was still present after the 6-month exposure-free period. The macrocytic anaemia observed at 700 ml/m<sup>3</sup> and the atrophy of skeletal muscles and testes at 7000 ml/m<sup>3</sup> were reversible after six months. Increased mortality occurred at 7000 ml/m<sup>3</sup> also during the recovery period.

Degeneration of the spinal cord occurred at 70 ml/m<sup>3</sup> and above and degeneration of the olfactory epithelium in the turbinates at 220 ml/m<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup>. Tumours are described in Section 5.7.2.

In a study with male and female B6C3F<sub>1</sub> 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<sup>3</sup> and above. At 70 ml/m<sup>3</sup> (males) or 280 ml/m<sup>3</sup> (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<sub>1</sub> mice at isoprene concentrations of 0, 70, 700 and 7000 ml/m<sup>3</sup>, 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<sup>3</sup>. 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<sup>3</sup> and above in the male mice. Decreased absolute testis weight and testicular atrophy occurred at 7000 ml/m<sup>3</sup>. At this concentration, oestrous cycle length was increased in the females. Therefore, the NOAEC for changes in the reproductive organs is 70 ml/m<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup> 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   |
|---|--|--|--|
| <b>rat</b> ,<br>no data on<br>strain,<br>♂,<br>no data on<br>number | <b>4 hours</b> ,<br>4000 mg/m <sup>3</sup><br>(about 1434 ml/m <sup>3</sup> ),<br>whole-body expo-<br>sure, controls: no<br>data   | <b>1434 ml/m<sup>3</sup></b> : no effects on spermatozoa 24 h<br>after end of exposure (number of spermatozoa,<br>percentage of living, motile and pathological<br>spermatozoa; no further details)  | Repina<br>1988                                     |
| <b>rat</b> ,<br>F344/N,<br>10 ♂/♀ per<br>group                      | <b>13 weeks</b> ,<br>0, 70, 700, 7000 ml/<br>m <sup>3</sup> , whole-body<br>exposure,<br>6 h/day, 5 days/wk,<br>purity: > 99%  | <b>7000 ml/m<sup>3</sup></b> : NOAEC<br>(sperm motility, vaginal cytology; compare<br>Section 5.2.1)   | Melnick<br>et al. 1994;<br>NTP 1995                |
| <b>rat</b> ,<br>F344/N,<br>40 ♂ per<br>group                        | <b>6 months</b> ,<br>0, 70, 220, 700, 2200,<br>7000 ml/m <sup>3</sup> ,<br>6 h/day, 5 days/wk,<br>per group 10 animals<br>investigated;<br>recovery period<br>6 months<br>(per group 30 ♂),<br>purity: > 99% | <b>after 6 months</b> :<br><b>7000 ml/m<sup>3</sup></b> : hyperplasia of interstitial cells of<br>the testes (10/10, control: 1/10);<br><b>6-month exposure and 6-month recovery<br/>period</b> :<br><b>at 700 ml/m<sup>3</sup> and above</b> : hyperplasia of inter-<br>stitial cells of the testes (30/30, control: 25/30);<br><b>7000 ml/m<sup>3</sup></b> : interstitial cell adenomas of the<br>testes;<br>see Section 5.2.1, Section 5.7.2 | Melnick<br>et al. 1992,<br>1994, 1996;<br>NTP 1995 |
| <b>mouse</b> ,<br>B6C3F <sub>1</sub> ,<br>10 ♂/♀ per<br>group       | <b>13 weeks</b> ,<br>0, 70, 700, 7000 ml/<br>m <sup>3</sup> , whole-body<br>exposure,<br>6 h/day, 5 days/wk,<br>purity: > 99%  | <b>70 ml/m<sup>3</sup></b> : NOAEC;<br><b>at 700 ml/m<sup>3</sup> and above</b> : ♂: absolute weight<br>of epididymides and cauda epididymides<br>decreased, sperm motility, sperm concentra-<br>tion, number of spermatids, sperm head<br>count per testes decreased;<br><b>7000 ml/m<sup>3</sup></b> : ♂: absolute testis decreased, testi-<br>cular atrophy (2/10), ♀: length of oestrous cycle<br>increased; see Section 5.2.1               | Melnick<br>et al. 1994;<br>NTP 1995                |
| <b>mouse</b> ,<br>B6C3F <sub>1</sub> ,<br>40 ♂ per<br>group         | <b>6 months</b> ,<br>0, 70, 220, 700, 2200,<br>7000 ml/m <sup>3</sup> ,<br>6 h/day, 5 days/wk,<br>per group 10 animals<br>investigated;<br>recovery period<br>6 months (per<br>group 30 ♂),<br>purity: > 99% | <b>after 6 months</b> :<br><b>7000 ml/m<sup>3</sup></b> : absolute and relative testicular<br>weight decreased, testicular atrophy;<br><b>6 months exposure and 6 months recovery<br/>period</b> :<br><b>7000 ml/m<sup>3</sup></b> : testicular weight and histo-<br>pathology of the testes without abnormal<br>findings;<br>see Section 5.2.1  | Melnick<br>et al. 1992,<br>1994, 1996;<br>NTP 1995 |



**Table 4** (Continued)

| Species  | Exposure  | Concentration: findings  | References           |
|--|---|--|----------------------|
| <b>mouse,</b><br>B6C3F <sub>1</sub> ,<br>10 ♀ per<br>group | <b>30 days,</b><br>0, 7.34 mmol/kg<br>body weight and d<br>(about 500 mg/kg<br>body weight and<br>day),<br>intraperitoneal, once/<br>day, controls: sesame<br>oil | <b>500 mg/kg body weight and day:</b> number of<br>small and growing follicles decreased | Doerr et al.<br>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<br>strain, no.<br>of animals                      | Exposure   | Concentration or dose: findings  | Referen-<br>ces                  |
|---|--|--|----------------------------------|
| <b>rat,</b><br>Sprague<br>Dawley,<br>24–26 ♀<br>per group | <b>gestation days 6–19,</b><br>0, 280, 1400, 7000 ml/<br>m <sup>3</sup> whole-body expo-<br>sure,<br>6 h, 7 days/wk,<br>investigated on<br>gestation day 20,<br>purity: >99% | <b>7000 ml/m<sup>3</sup>:</b><br><u>foetuses:</u> NOAEC;<br><u>dams:</u> NOAEC for maternal toxicity (with<br>regard to mortality and body weight gain)  | Mast et al.<br>1990; NTP<br>1995 |
| <b>mouse,</b><br>CD-1 Swiss,<br>28–30 ♀ per<br>group      | <b>gestation days 6–17,</b><br>0, 280, 1400, 7000 ml/<br>m <sup>3</sup> whole-body expo-<br>sure,<br>6 h, 7 days/wk,<br>investigated on<br>gestation day 18,<br>purity: >99% | <b>at 280 ml/m<sup>3</sup> and above:</b><br><u>foetuses:</u> ♀ foetus weight decreased, NOAEC;<br><b>1400 ml/m<sup>3</sup>:</b><br><u>dams:</u> NOAEC for maternal toxicity;<br><b>at 1400 ml/m<sup>3</sup> and above:</b><br><u>foetuses:</u> ♂ foetuses weight decreased;<br><b>7000 ml/m<sup>3</sup>:</b><br><u>foetuses:</u> foetuses per litter with variations/re-<br>duced ossification (mostly supernumerary ribs)<br>increased;<br><u>dams:</u> body weight gain decreased, uterus<br>weight (gravid uterus) decreased, absolute and<br>relative kidney weight increased | Mast et al.<br>1990; NTP<br>1995 |

**Table 6** (Continued)

| Species strain, no. of animals | Exposure  | Concentration or dose: findings  | References   |
|--------------------------------|---|--|--------------|
| rat, Wistar, 10 ♀ per group    | <b>gestation days 9–12</b> , at 22 mg/kg body weight and above:<br>0, 22, 379, 1895 mg/kg body weight and day, oral (no further details), time of study: no data, purity: no data | <b>at 22 mg/kg body weight and above:</b><br><u>foetuses</u> : number of resorptions increased (not dose-dependently), reduced ossification of the sternum and of the occipital bone increased (also not dose-dependently);<br><b>up to 1895 mg/kg body weight:</b><br><u>foetuses</u> : no skeletal and no external malformations;<br><u>dams</u> : 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<sup>3</sup> from days 6 to 19 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<sup>3</sup> (Mast et al. 1990; NTP 1995). A NOAEC of 7000 ml/m<sup>3</sup> is thus assumed for maternal toxicity and developmental toxicity from this study.

After inhalation of isoprene at 0, 280, 1400 or 7000 ml/m<sup>3</sup> 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<sup>3</sup>. 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<sup>3</sup> and above. The percentage of foetuses per litter with variations/reduced ossification (mostly supernumerary ribs) was increased at 7000 ml/m<sup>3</sup> (see Table 7).

A concentration of 1400 ml/m<sup>3</sup> 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<sub>1</sub> mice lasting 13 weeks, a NOAEC of 220 ml/m<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup>.

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

| Endpoint   | Isoprene concentration (ml/m <sup>3</sup> ) |             |              |              |              |
|--|---|-------------|--------------|--------------|--------------|
|  | 0   | 280         | 1400         | 7000         |              |
| Number of investigated dams/litters  | 28  | 29          | 28           | 27           |              |
| Living fetuses/litter (M±SD)   | 11.5 ± 3.0                                  | 12.0 ± 1.9  | 11.9 ± 2.2   | 10.9 ± 1.8   |              |
| Dead fetuses/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 <sup>a)</sup> (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);<sup>a)</sup> 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 fetuses was reduced in the low, but not in the high dose groups. Therefore, no substance-specific effect is to be assumed. In the fetuses 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 fetuses 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;<br>purity;<br>solvent                                       | Results |        | Cyto<br>toxicity    | References                |
|--------------------------------------|--|--|---------|--------|---------------------|---------------------------|
|                                      |  |  | – m.a.  | + m.a. |                     |                           |
| BMT                                  | <i>S. typhimurium</i><br>TA102, TA104                              | no further details;<br>gas phase   | –       | n.p.   | no data             | Kushi et al.<br>1985      |
| BMT                                  | <i>S. typhimurium</i><br>TA98, TA100,<br>TA1530, TA1535,<br>TA1538 | 25% v/v (TA1530<br>with 75% isoprene<br>v/v); 99%;<br>gas phase            | –       | –      | no data             | de Meester<br>et al. 1981 |
| BMT<br>(preincu-<br>bation)          | <i>S. typhimurium</i><br>TA98, TA100,<br>TA1535, TA1537            | 100–10 000<br>µg/plate;<br>> 99%;<br>DMSO                                  | –       | –      | 10 000 µg/<br>plate | Mortelmans<br>et al. 1986 |
| BMT<br>(plate<br>incorpora-<br>tion) | <i>S. typhimurium</i><br>TA98, TA100,<br>TA1535, TA1537            | 100–10 000<br>µg/plate;<br>> 99%;<br>DMSO                                  | –       | –      | 10 000 µg/<br>plate | NTP 1983,<br>1995, 1999   |
| SCE                                  | CHO cells  | 50–1600 µg/ml<br>(–m.a.);<br>160–5000 µg/ml<br>(+ m.a.);<br>> 99%;<br>DMSO | –       | –      | no data             | NTP 1995,<br>1999         |
| CA                                   | CHO cells  | 1600–5000 µg/ml;<br>> 99%;<br>DMSO   | –       | –      | no data             | NTP 1995,<br>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;<br>purity;<br>solvent                                       | Results                                       |        | Cyto<br>toxicity              | References             |
|---------------------------------|--------------------------------------|--|---|--------|-------------------------------|------------------------|
|                                 |                                      |  | – m.a.  | + m.a. |                               |                        |
| BMT<br>(plate<br>incorporation) | <i>S. typhimurium</i><br>TA98, TA100 | 7.5–30 mM 1,2-<br>epoxy-2-methyl-3-<br>butene;<br>95%;<br>DMSO             | –   | n. p.  | 30 mM<br>(TA100),<br>– (TA98) | Gervasi<br>et al. 1985 |
| BMT<br>(plate<br>incorporation) | <i>S. typhimurium</i><br>TA98, TA100 | 5–30 mM 1,2-<br>epoxy-3-methyl-3-<br>butene;<br>no data on purity;<br>DMSO | –   | n. p.  | 30 mM                         | Gervasi<br>et al. 1985 |
| BMT<br>(plate<br>incorporation) | <i>S. typhimurium</i><br>TA98, TA100 | 2–30 mM 1,2:3,4-<br>diepoxy-2-methyl<br>butane;<br>99%;<br>DMSO            | + at 7.5<br>mM/plate<br>and above<br>in TA100 | n. p.  | 30 mM                         | Gervasi<br>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 diepoxy-ide 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<sup>3</sup> and above; no further increase in SCE frequencies was observed at about 700 ml/m<sup>3</sup> and above (Shelby 1990; Tice 1988; Tice et al. 1988).

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

| Test system    |   | Duration,<br>mode of<br>administration,<br>concentration   | Results   | References   |
|----------------|---|--|---|--|
| SCE            | mouse,<br>B6C3F <sub>1</sub> ,<br>4 ♂ per<br>group  | <b>12 days</b> ,<br>inhalation,<br>0, 438, 1750,<br>7000 ml/m <sup>3</sup> ;<br>6 h/day (3 days expo-<br>sure, 2 days expo-<br>sure-free, 5 days<br>exposure, 2 days<br>exposure-free,<br>4 days exposure) | 17–20 h after end of exposure:<br>bone marrow cells:<br>+ at and above 438 ml/m <sup>3</sup><br>bone marrow cells: average genera-<br>tion time at 7000 ml/m <sup>3</sup> increased;<br>mitotic index unchanged   | Tice 1988;<br>Tice et al.<br>1988                          |
| SCE            | mouse,<br>B6C3F <sub>1</sub> , 4 ♂<br>per group     | <b>12 days</b> ,<br>inhalation,<br>0, 70, 220, 700 ml/<br>m <sup>3</sup> ; 6 h/day (3 days<br>exposure, 2 days<br>exposure-free, 5 days<br>exposure, 2 days<br>exposure-free,<br>4 days exposure)          | 17–20 h after end of exposure: bone<br>marrow cells:<br>+ at and above 220 ml/m <sup>3</sup> , concentra-<br>tion-dependent   | Shelby<br>1990   |
| muta-<br>tions | mouse,<br>B6C3F <sub>1</sub> ,<br>40 ♂ per<br>group | <b>26 weeks</b> ,<br>inhalation,<br>0, 2200, 7000 ml/m <sup>3</sup> ;<br>6 h/day, 5 days/wk,<br>recovery period:<br>26 w   | in tumours of <b>Harderian gland</b> :<br>60% K-ras-mutations and 40% H-<br>ras-mutations (spontaneous tu-<br>mours in controls: 8% K-ras and<br>48% H-ras); A→T transversions in<br>K-ras codon 61: 15/30 and C→A<br>transversions in H-ras codon 61: 8/<br>30 (spontaneous tumours in con-<br>trols: 2/27 K-ras and 4/25<br>H-ras);<br>increased proliferating cell nuclear<br>antigen index in the tumours<br>compared with spontaneous tumours;<br>in <b>lung</b> tumours:<br>A→T transversions at K-ras codon 61:<br>10/11 (spontaneous tumours in con-<br>trols: 0/82 K-ras);<br>in <b>forestomach</b> tumours:<br>G→C transversions at K-ras codon 13:<br>5/10 and A→T transversions at H-ras<br>codon 61: 2/10 (spontaneous tumours<br>in controls: 1/11 K-ras and 0/11<br>H-ras) | Hong et al.<br>1997; Sills<br>et al.<br>1999 a, b,<br>2001 |

**Table 10** (Continued)

| Test system |  | Duration,<br>mode of<br>administration,<br>concentration  | Results   | References  |
|-------------|--|---|---|---|
| MN          | rat, F344/N,<br>10 ♂, ♀ per<br>group                   | <b>4 weeks,</b><br>inhalation,<br>0, 220, 700,<br>7000 ml/m <sup>3</sup> ;<br>6 h/day, 5 days/wk;<br>purity: > 99%<br>no positive controls  | lung fibroblasts:<br>–  | NTP 1999  |
| MN          | mouse,<br>B6C3F <sub>1</sub> ,<br>15 ♂ per<br>group    | <b>12 days,</b><br>inhalation,<br>0, 438, 1750,<br>7000 ml/m <sup>3</sup> ;<br>6 h/day (3 days expo-<br>sure, 2 days expo-<br>sure-free, 5 days<br>exposure, 2 days<br>exposure-free,<br>4 days exposure) | PCEs, NCEs in peripheral blood:<br>+ at and above 438 ml/m <sup>3</sup> 23 h after<br>end of exposure   | Shelby and<br>Witt 1995;<br>Tice 1988;<br>Tice et al.<br>1988 |
| MN          | mouse,<br>B6C3F <sub>1</sub> ,<br>15 ♂ per<br>group    | <b>12 days,</b><br>inhalation,<br>0, 70, 220, 700 ml/<br>m <sup>3</sup> ; 6 h/day (3 days<br>exposure, 2 days ex-<br>posure-free, 5 days<br>exposure, 2 days<br>exposure-free,<br>4 days exposure)        | PCEs, NCEs in peripheral blood:<br>+ at 700 ml/m <sup>3</sup> 23 h after end of<br>exposure   | Shelby<br>1990  |
| MN          | mouse,<br>B6C3F <sub>1</sub> ,<br>10 ♂, ♀ per<br>group | <b>13 weeks,</b><br>inhalation,<br>0, 70, 220, 700, 2200,<br>7000 ml/m <sup>3</sup> ;<br>6 h/day, 5 days/wk;<br>purity: > 99%<br>no positive controls   | ♂: PCEs, NCEs in peripheral blood:<br>+ at 700 ml/m <sup>3</sup> and above;<br>♀: PCEs, NCEs in peripheral blood:<br>+ at 220 ml/m <sup>3</sup> and above | NTP 1999  |
| MN          | mouse,<br>B6C3F <sub>1</sub> , 10 ♂<br>per group       | <b>40 weeks,</b><br>inhalation,<br>0, 70, 140,<br>2200 ml/m <sup>3</sup> ;<br>8 h/day, 5 days/wk  | 24 h after end of exposure:<br>PCEs in peripheral blood:<br>+ at 2200 ml/m <sup>3</sup>   | Placke<br>et al. 1996   |

**Table 10** (Continued)

| Test system |  | Duration,<br>mode of<br>administration,<br>concentration   | Results  | References  |
|-------------|--|--|--|---|
| MN          | mouse,<br>B6C3F <sub>1</sub><br>10 ♂ per<br>group  | <b>80 weeks</b> ,<br>inhalation,<br>0, 10, 70, 280, 700,<br>2200 ml/m <sup>3</sup> ;<br>8 h/day (at 2200 ml/m <sup>3</sup><br>additional: 4 h/day),<br>5 days/wk   | 24 h after end of exposure:<br>PCEs in peripheral blood:<br>+ at and above 700 ml/m <sup>3</sup> (also at<br>2200 ml/m <sup>3</sup> after 4 h) | Placke<br>et al. 1996   |
| CA          | mouse,<br>B6C3F <sub>1</sub> ,<br>8 ♂ per<br>group | <b>12 days</b> ,<br>inhalation,<br>0, 438, 1750,<br>7000 ml/m <sup>3</sup> ;<br>6 h/day (3 days expo-<br>sure, 2 days expo-<br>sure-free, 5 days ex-<br>posure, 2 days expo-<br>sure-free,<br>4 days exposure) | 17–20 h after end of exposure: bone<br>marrow cells:<br>–  | Shelby and<br>Witt 1995;<br>Tice 1988;<br>Tice et al.<br>1988 |
| CA          | mouse,<br>B6C3F <sub>1</sub> ,<br>8 ♂ per<br>group | <b>12 days</b> ,<br>inhalation,<br>0, 70, 220, 700 ml/<br>m <sup>3</sup> ; 6 h/day (3 days<br>exposure, 2 days ex-<br>posure-free, 5 days<br>exposure, 2 days ex-<br>posure-free,<br>4 days exposure)          | 17–20 h after end of exposure: bone<br>marrow cells:<br>–  | Shelby<br>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<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup>. 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<sup>3</sup> 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:   | <b>rat</b> 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 <sup>3</sup>   |                  |                |                  |                  |                   |
| Duration:  | 26 wk, 6 h/day, 5 days/wk, recovery period: 26 wk   |                  |                |                  |                  |                   |
| Toxicity:  | None  |                  |                |                  |                  |                   |
|  | Isoprene (ml/m <sup>3</sup> )   |                  |                |                  |                  |                   |
|  | 0   | 70               | 220            | 700              | 2200             | 7000              |
| <b>Tumours and preneoplasias</b>   |   |                  |                |                  |                  |                   |
| <b>Testes:</b>   |   |                  |                |                  |                  |                   |
| hyperplasia of interstitial cells after 26 weeks   | 1/10<br>(10%)   | 1/10<br>(10%)    | 3/10<br>(30%)  | 1/10<br>(10%)    | 3/10<br>(30%)    | 10/10<br>(100%)** |
| hyperplasia of interstitial cells after 52 weeks   | 25/30<br>(83%)  | 30/30<br>(100%)* | 28/30<br>(93%) | 30/30<br>(100%)* | 29/29<br>(100%)* | 30/30<br>(100%)*  |
| adenomas of interstitial cells, including multiple adenomas  | 3/30<br>(10%)   | 3/30<br>(10%)    | 4/30<br>(13%)  | 7/30<br>(23%)    | 8/29<br>(28%)    | 9/30<br>(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:   | <b>rat</b> F344/N, 50 ♂/♀ per group   |                  |                |                  |                  |                   |
| Application:   | inhalation, whole-body exposure   |                  |                |                  |                  |                   |
| Concentration:   | 0, 220, 700, 7000 ml/m <sup>3</sup>   |                  |                |                  |                  |                   |
| Duration:  | 105 wk, 6 h/day, 5 days/wk  |                  |                |                  |                  |                   |
| Toxicity:  | <b>at 700 ml/m<sup>3</sup> and above:</b> ♂: splenic fibrosis, renal tubular hyperplasia;<br><b>7000 ml/m<sup>3</sup>:</b> ♂: hyperplasia in the parathyroid gland;<br>♀: suppurative inflammation in the nose, bile duct hyperplasia |                  |                |                  |                  |                   |
|  | Isoprene (ml/m <sup>3</sup> )   |                  |                |                  |                  |                   |
|  | 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   |                 |             |               |               |               |
|---|-----------------|-------------|---------------|---------------|---------------|
| <b>Kidney:</b>  |                 |             |               |               |               |
| renal tubule, hyperplasia<br>(standard evaluation) <sup>1)</sup>              | ♂               | 0/50 (0%)   | 2/50 (4%)     | 6/50 (12%)*   | 8/50 (16%)**  |
| renal tubule, hyperplasia<br>(standard and extended evaluation) <sup>1)</sup> | ♂               | 7/50(14%)   | 6/50(12%)     | 13/50 (26%)   | 18/50 (36%)** |
| renal tubule adenoma<br>(standard evaluation) <sup>1)</sup>                   | ♂               | 0/50 (0%)   | 2/50 (4%)     | 2/50 (4%)     | 6/50 (12%)*   |
| renal tubule adenoma<br>(standard and extended<br>evaluation) <sup>1)</sup>   | ♂               | 2/50 (4%)   | 4/50 (8%)     | 8/50 (16%)*   | 15/50 (30%)** |
| <b>Mammary gland:</b>   |                 |             |               |               |               |
| 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   | ♂ <sup>2)</sup> | 2/50 (4%)   | 4/50 (8%)     | 6/50 (12%)    | 21/50 (42%)** |
|   | ♀ <sup>2)</sup> | 19/50 (38%) | 35/50 (70%)** | 32/50 (64%)** | 32/50 (64%)** |
| carcinoma   | ♂ <sup>2)</sup> | 0/42 (0%)   | 1/43 (2%)     | 1/47 (2%)     | 2/44 (5%)     |
|   | ♀               | 4/50 (8%)   | 2/50 (4%)     | 1/50 (2%)     | 3/50 (6%)     |
| <b>Testes:</b>  |                 |             |               |               |               |
| interstitial cell adenoma, bilateral  | ♂               | 20/50 (40%) | 29/50 (58%)   | 37/50 (74%)** | 48/50 (96%)** |
| interstitial cell adenoma, unilateral<br>and bilateral                        | ♂ <sup>3)</sup> | 33/50 (66%) | 37/50 (74%)   | 44/50 (88%)*  | 48/50 (96%)** |
| <b>Nervous system/brain:</b>  |                 |             |               |               |               |
| benign astrocytoma  | ♀ <sup>4)</sup> | 0/50 (0%)   | 0/50 (0%)     | 1/50 (2%)     | 0/50 (0%)     |
| malignant astrocytoma   | ♂ <sup>5)</sup> | 0/50 (0%)   | 0/50 (0%)     | 0/50 (0%)     | 1/50 (2%)     |
| malignant glioma  | ♀ <sup>6)</sup> | 0/50 (0%)   | 0/50 (0%)     | 0/50 (0%)     | 1/50 (2%)     |
| malignant medulloblastoma   | ♀ <sup>7)</sup> | 0/50 (0%)   | 0/50 (0%)     | 0/50 (0%)     | 1/50 (2%)     |
| meninges, benign granular cell tumour   | ♂ <sup>8)</sup> | 0/50 (0%)   | 0/50 (0%)     | 1/50 (2%)     | 0/50 (0%)     |
|   | ♀ <sup>8)</sup> | 0/50 (0%)   | 1/50 (2%)     | 0/50 (0%)     | 1/50(2%)      |
| meninges, sarcoma   | ♀ <sup>9)</sup> | 0/50 (0%)   | 1/50(2%)      | 0/50 (0%)     | 1/50 (2%)     |
| <b>Lymphohaematopoietic system:</b>   |                 |             |               |               |               |
| mononuclear leukaemia   | ♀               | 14/50 (28%) | 15/50 (30%)   | 21/50 (42%)   | 21/50 (42%)   |

\*p ≤ 0.05; \*\*p ≤ 0.01 (Fisher's exact test)

<sup>1)</sup>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 kidney. Historical control data of the laboratory: total incidence (average incidence per test ± standard deviation; range)<sup>2)</sup>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%);<sup>3)</sup>Testicular tumours ♂: 628/905 (69.4±9.7%; 46–83%);<sup>4)</sup>Benign astrocytoma ♀: 1/899 (0.1 ±0.5%; 0–2%);<sup>5)</sup>Malignant astrocytoma ♂: 1/904 (0.1 ±0.5%; 0–2%);<sup>6)</sup>Malignant glioma ♀: 1/899 (0.1 ±0.5%; 0–2%);<sup>7)</sup>Malignant medulloblastoma ♀: 0/899;<sup>8)</sup>Benign granular cell tumour of the meninges ♂: 0/904; ♀ : 2/899 (0.2±0.7%; 0–2%);<sup>9)</sup>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 <sub>1</sub> , 40 ♂ per group (10 per group for interim sacrifice)  |               |               |                   |                                 |                   |
| Administration:                          | inhalation, whole-body exposure   |               |               |                   |                                 |                   |
| Concentration:                           | 0, 70, 220, 700, 2200, 7000 ml/m <sup>3</sup>   |               |               |                   |                                 |                   |
| Duration:                                | 26 wk, 6 h/day, 5 days/wk, recovery period: 26 wk   |               |               |                   |                                 |                   |
| Toxicity:                                | <b>After 26 weeks:</b><br><b>at 220 ml/m<sup>3</sup> and above:</b> grip strength of fore- and hindlimbs decreased<br><b>at 700 ml/m<sup>3</sup> and above:</b> epithelial hyperplasia of the forestomach, macrocytic anaemia;<br><b>7000 ml/m<sup>3</sup>:</b> degeneration of the olfactory epithelium in the turbinates, spinal cord degeneration, atrophy of skeletal muscles, testicular atrophy<br><b>after 52 weeks:</b><br><b>at 70 ml/m<sup>3</sup> and above:</b> spinal cord degeneration ;<br><b>at 700 ml/m<sup>3</sup> and above:</b> degeneration of the olfactory epithelium in the turbinates, epithelial hyperplasia of the forestomach;<br><b>at 2200 ml/m<sup>3</sup> and above:</b> hyperplasia of the alveolar epithelium;<br><b>7000 ml/m<sup>3</sup>:</b> mortality increased, testicular atrophy |               |               |                   |                                 |                   |
| <hr/>                                    |   |               |               |                   |                                 |                   |
|  | Isoprene (ml/m <sup>3</sup> )   |               |               |                   |                                 |                   |
|  | 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*            |
| <hr/>                                    |   |               |               |                   |                                 |                   |
| <b>Tumours and preneoplastic lesions</b> |   |               |               |                   |                                 |                   |
| <hr/>                                    |   |               |               |                   |                                 |                   |
| <b>Harderian gland:</b>                  |   |               |               |                   |                                 |                   |
| hyperplasia                              | 1/30<br>(3%)  | 0/30<br>(0%)  | 2/29<br>(7%)  | 2/30<br>(7%)      | 2/30<br>(7%)                    | 2/28<br>(7%)      |
| adenoma                                  | 2/30<br>(7%)  | 6/30<br>(20%) | 4/30<br>(13%) | 14/30<br>(47%)*** | 13/30 <sup>1)</sup><br>(43%)*** | 12/30<br>(40%)*** |
| <hr/>                                    |   |               |               |                   |                                 |                   |
| <b>Liver:</b>                            |   |               |               |                   |                                 |                   |
| basophilic foci                          | 3/30<br>(10%)   | 1/30<br>(3%)  | 1/29<br>(3%)  | 2/30<br>(7%)      | 5/30<br>(17%)                   | 3/28<br>(11%)     |
| eosinophilic foci                        | 1/30<br>(3%)  | 0/30<br>(0%)  | 0/29<br>(0%)  | 6/30<br>(20%)     | 5/30<br>(17%)                   | 3/28<br>(11%)     |
| mixed foci                               | 0/30<br>(0%)  | 0/30<br>(0%)  | 1/29<br>(3%)  | 1/30<br>(3%)      | 2/30<br>(7%)                    | 3/28<br>(11%)     |
| hepatocellular adenoma                   | 4/30<br>(13%)   | 2/30<br>(7%)  | 6/29<br>(21%) | 15/30<br>(50%)*** | 18/30<br>(60%)***               | 16/28<br>(57%)*** |
| hepatocellular carcinoma                 | 4/30<br>(13%)   | 1/30<br>(3%)  | 3/29<br>(10%) | 5/30<br>(17%)     | 4/30<br>(13%)                   | 9/28<br>(32%)*    |
| hepatocellular adenoma and carcinoma     | 7/30<br>(23%)   | 3/30<br>(10%) | 7/29<br>(24%) | 15/30<br>(50%)*   | 18/30<br>(60%)***               | 17/28<br>(61%)*** |

**Table 11** (Continued)

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

\*  $p < 0.05$  (logistic regression test); \*\*  $p < 0.01$  (Fisher's exact test); \*\*\*  $p < 0.01$  (logistic regression test)

<sup>†</sup> one mouse had an additional carcinoma

|                 |  |
|-----------------|--|
| Author:         | Cox et al. 1996; Placke et al. 1996  |
| Substance:      | Isoprene $\geq 99\%$ , $<1\%$ limonene (tert-butyl catechol as stabilizer (concentration: 50 ppm)  |
| Species:        | <b>mouse</b> B6C3F <sub>1</sub> , 50 ♂ per group   |
| Administration: | inhalation; whole-body exposure  |
| Concentration:  | 0, 10, 70, 140, 280, 700, 2200 ml/m <sup>3</sup>   |
| Duration:       | 8 h/day, 5 days/wk<br>20 w: 0, 280, 2200 ml/m <sup>3</sup> ; (2200 ml/m <sup>3</sup> , 4 h)<br>40 w: 0, 70, 140, 2200 ml/m <sup>3</sup><br>80 w: 0, 10, 70, 280, 700, 2200 ml/m <sup>3</sup> (2200 ml/m <sup>3</sup> , 4 and 8 h)  |
| Toxicity:       | recovery period: up to week 104 each<br><b>at 10 ml/m<sup>3</sup> and above:</b> proliferation of haematopoietic cells in the spleen, myeloid hyperplasia of the bone marrow;<br><b>at 280 ml/m<sup>3</sup> and above:</b> survival rate after 80 weeks exposure $< 50\%$ (no further details), mild metaplasia of the olfactory epithelium to respiratory epithelium;<br><b>at higher concentrations</b> (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 <sup>3</sup> )                   |                |                |                  |                  |                  |                  |
|                                     | 0   | 10             | 70             | 280              | 700              | 2200<br>(4h)     | 2200<br>(8h)     |
|                                     | Cumulative exposure (ml/m <sup>3</sup> × weeks) |                |                |                  |                  |                  |                  |
|                                     | 0   | 800            | 5600           | 22 400           | 56 000           | 88 000           | 176 240          |
| <b>Harderian gland:</b>             |   |                |                |                  |                  |                  |                  |
| adenoma                             | 4/47<br>(8%)                                    | 4/49<br>(8%)   | 9/50<br>(18%)  | 17/50<br>(34%)** | 26/49<br>(52%)** | 28/50<br>(56%)** | 35/50<br>(70%)** |
| carcinoma                           | 0/47<br>(0%)                                    | 0/49<br>(0%)   | 0/50<br>(0%)   | 1/50<br>(2%)     | 3/49<br>(6%)     | 2/50<br>(4%)     | 2/50<br>(4%)     |
| <b>Liver:</b>                       |   |                |                |                  |                  |                  |                  |
| hepatocellular adenoma              | 11/50<br>(22%)                                  | 12/50<br>(24%) | 15/50<br>(30%) | 24/50<br>(48%)** | 27/48<br>(56%)** | 21/50<br>(42%)** | 30/50<br>(60%)** |
| hepatocellular carcinoma            | 9/50<br>(18%)                                   | 6/50<br>(12%)  | 9/50<br>(18%)  | 16/50<br>(32%)   | 17/48<br>(35%)*  | 15/50<br>(30%)   | 16/50<br>(32%)   |
| <b>Lung:</b>                        |   |                |                |                  |                  |                  |                  |
| alveolar/bronchiolar adenoma        | 11/50<br>(22%)                                  | 16/50<br>(32%) | 4/50<br>(8%)   | 13/50<br>(26%)   | 23/50<br>(46%)** | 15/50<br>(30%)   | 30/50<br>(60%)** |
| alveolar/bronchiolar carcinoma      | 0/50<br>(0%)                                    | 1/50<br>(2%)   | 2/50<br>(4%)   | 1/50<br>(2%)     | 7/50<br>(14%)**  | 3/50<br>(6%)     | 7/50<br>(14%)**  |
| <b>Spleen:</b>                      |   |                |                |                  |                  |                  |                  |
| haemangiosarcoma                    | 1/49<br>(2%)                                    | 3/48<br>(6%)   | 2/50<br>(4%)   | 1/50<br>(2%)     | 2/48<br>(4%)     | 2/50<br>(4%)     | 1/49<br>(2%)     |
| <b>Heart:</b>                       |   |                |                |                  |                  |                  |                  |
| haemangiosarcoma                    | 0/49<br>(0%)                                    | 0/50<br>(0%)   | 0/50<br>(0%)   | 2/50<br>(4%)     | 1/50<br>(2%)     | 1/50<br>(2%)     | 1/50<br>(2%)     |
| <b>Forestomach:</b>                 |   |                |                |                  |                  |                  |                  |
| squamous cell papilloma             | 0/50<br>(0%)                                    | 0/48<br>(0%)   | 0/50<br>(0%)   | 0/50<br>(0%)     | 1/47<br>(2%)     | 1/50<br>(2%)     | 1/50<br>(2%)     |
| squamous cell carcinoma             | 0/50<br>(0%)                                    | 0/48<br>(0%)   | 0/50<br>(0%)   | 1/50<br>(2%)     | 0/47<br>(0%)     | 1/50<br>(2%)     | 3/50<br>(6%)     |
| <b>Lymphohaematopoietic system:</b> |   |                |                |                  |                  |                  |                  |
| histiocytic sarcoma                 | 0/50<br>(0%)                                    | 2/50<br>(4%)   | 2/50<br>(4%)   | 4/50<br>(8%)     | 2/50<br>(4%)     | 7/50<br>(14%)**  | 2/50<br>(4%)     |
| lymphoma                            | 2/50<br>(4%)                                    | 1/50<br>(2%)   | 4/50<br>(8%)   | 5/50<br>(10%)    | 4/50<br>(8%)     | 4/50<br>(8%)     | 6/50<br>(12%)    |
| 40-week exposure                    |   |                |                |                  |                  |                  |                  |
|                                     | Isoprene (ml/m <sup>3</sup> )                   |                |                |                  |                  |                  |                  |
|                                     | 0   | 70             | 140            | 2200             |                  |                  |                  |
|                                     | Cumulative exposure (ml/m <sup>3</sup> × weeks) |                |                |                  |                  |                  |                  |
|                                     | 0   | 2800           | 5600           | 88 000           |                  |                  |                  |
| <b>Harderian gland:</b>             |   |                |                |                  |                  |                  |                  |
| 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)

|                                     |             |                                     |               |               |
|-------------------------------------|-------------|-------------------------------------|---------------|---------------|
| <b>Liver:</b>                       |             |                                     |               |               |
| 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%)*   |
| <b>Lung:</b>                        |             |                                     |               |               |
| 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%)     |
| <b>Spleen:</b>                      |             |                                     |               |               |
| haemangiosarcoma                    | 1/49 (2%)   | 1/47 (2%)                           | 3/50 (6%)     | 0/47 (0%)     |
| <b>Heart:</b>                       |             |                                     |               |               |
| haemangiosarcoma                    | 0/49 (0%)   | 0/49 (0%)                           | 0/50 (0%)     | 1/49 (2%)     |
| <b>Forestomach:</b>                 |             |                                     |               |               |
| squamous cell papilloma             | 0/50 (0%)   | 0/47 (0%)                           | 0/49 (0%)     | 2/47 (4%)     |
| <b>Lymphohaematopoietic system:</b> |             |                                     |               |               |
| 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%)    |
| <hr/>                               |             |                                     |               |               |
| <b>20-week exposure</b>             |             | <hr/>                               |               |               |
|                                     |             | Isoprene (ml/m³)                    |               |               |
|                                     |             | 0                                   | 280           | 2200 (4 h)    |
|                                     |             | <hr/>                               |               |               |
|                                     |             | Cumulative exposure (ml/m³ × weeks) |               |               |
|                                     |             | 0                                   | 5600          | 22 000        |
| <hr/>                               |             |                                     |               |               |
| <b>Harderian gland:</b>             |             |                                     |               |               |
| adenoma                             |             | 4/47 (9%)                           | 16/49 (32%)** | 19/49 (40%)** |
| carcinoma                           |             | 0/47 (0%)                           | 3/49 (6%)     | 1/49 (2%)     |
| <hr/>                               |             |                                     |               |               |
| <b>Liver:</b>                       |             |                                     |               |               |
| hepatocellular adenoma              |             | 11/50 (22%)                         | 18/49 (37%)   | 22/50 (44%)*  |
| hepatocellular carcinoma            |             | 9/50 (18%)                          | 12/49 (25%)   | 12/50 (24%)   |
| <b>Lung:</b>                        |             |                                     |               |               |
| alveolar/bronchiolar adenoma        |             | 11/50 (22%)                         | 16/50 (32%)   | 14/50 (28%)   |
| alveolar/bronchiolar carcinoma      |             | 0/50 (0%)                           | 3/50 (6%)     | 2/50 (4%)     |
| <b>Spleen:</b>                      |             |                                     |               |               |
| haemangiosarcoma                    |             | 1/49 (2%)                           | 2/47 (4%)     | 2/48 (4%)     |
| <b>Heart:</b>                       |             |                                     |               |               |
| haemangiosarcoma                    |             | 0/49 (0%)                           | 0/49 (0%)     | 4/50 (8%)     |
| <b>Forestomach:</b>                 |             |                                     |               |               |
| squamous cell carcinoma             |             | 0/50 (0%)                           | 0/46 (0%)     | 1/50 (2%)     |
| <b>Lymphohaematopoietic system:</b> |             |                                     |               |               |
| histiocytic sarcoma                 |             | 0/50 (0%)                           | 8/50 (16%)**  | 5/50 (10%)*   |
| lymphoma                            |             | 2/50 (4%)                           | 7/50 (14%)    | 4/50 (8%)     |

\* p ≤ 0.05; \*\* p ≤ 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 <sub>1</sub> , 50 ♀ per group   |             |              |
| Administration:                     | inhalation, whole-body exposure   |             |              |
| Concentration:                      | 0, 10, 70 ml/m <sup>3</sup>   |             |              |
| Duration:                           | 80 wk, 8 h/day, 5 days/wk, recovery period: 24 wk   |             |              |
| Toxicity:                           | <b>at 10 ml/m<sup>3</sup> and above:</b> proliferation of haematopoietic cells in the spleen, myeloid hyperplasia of the bone marrow<br><b>70 ml/m<sup>3</sup>:</b> mild metaplasia of the olfactory epithelium to respiratory epithelium |             |              |
| <hr/>                               |   |             |              |
| <b>Tumours after 104 weeks:</b>     |   |             |              |
| <hr/>                               |   |             |              |
|                                     | Isoprene (ml/m <sup>3</sup> )   |             |              |
|                                     | 0   | 10          | 70           |
|                                     | Cumulative exposure (ml/m <sup>3</sup> × weeks)   |             |              |
|                                     | 0   | 800         | 5600         |
| <hr/>                               |   |             |              |
| <b>Harderian gland:</b>             |   |             |              |
| adenoma                             | 2/49 (4%)   | 3/49 (6%)   | 8/49(16%)*   |
| <b>Spleen:</b>                      |   |             |              |
| haemangiosarcoma                    | 1/50 (2%)   | 1/49 (2%)   | 4/50 (8%)    |
| <b>Pituitary gland:</b>             |   |             |              |
| adenoma                             | 1/49 (2%)   | 6/46 (13%)  | 9/49 (18%)** |
| <b>Lymphohaematopoietic system:</b> |   |             |              |
| 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<sup>3</sup> (whole-body exposure) for 105 weeks. The incidences of mammary fibroadenomas in females at 220 ml/m<sup>3</sup> and above, of renal tubule adenomas and of interstitial cell tumours of the testes in males at 770 ml/m<sup>3</sup> and above were significantly increased. The incidence of mammary fibroadenomas exceeded the range of historical controls in males at 220 ml/m<sup>3</sup> and above. The increase was concentration-dependent, and was significant at 7000 ml/m<sup>3</sup>. 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<sup>3</sup>.

### Mouse

In an NTP study, male B6C3F<sub>1</sub> mice were whole-body exposed to 0, 70, 220, 700, 2200 or 7000 ml isoprene/m<sup>3</sup> 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<sup>3</sup> and above, statistically significant increase at 700 ml/m<sup>3</sup> and above), hepatocellular adenomas and carcinomas (at 700 ml/m<sup>3</sup> and above), alveolar/bronchiolar adenomas and carcinomas (at 2200 ml/m<sup>3</sup> and above) and squamous cell papillomas and carcinomas of the forestomach (increase numerical at 2200 ml/m<sup>3</sup> and above, statistically significant at 7000 ml/m<sup>3</sup>). Mortality was increased by the end of the study at 2200 and 7000 ml/m<sup>3</sup> (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<sub>1</sub> 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<sup>3</sup> and above), hepatocellular adenomas (at 140 ml/m<sup>3</sup> and above) and carcinomas (at 700 ml/m<sup>3</sup> and above), alveolar/bronchiolar adenomas and carcinomas (700 ml/m<sup>3</sup>) and histiocytic sarcomas (280 ml/m<sup>3</sup>). Harderian gland adenomas already occurred in the animals exposed for only 20 weeks. Haemangiosarcomas in the heart (at 280 ml/m<sup>3</sup> and above) were observed in exposed male mice only, but were not statistically significant. This type of haemangiosarcoma is extremely rare in B6C3F<sub>1</sub> mice: in fact, none were found in 658 historical controls. In female mice, the incidence of haemangiosarcomas in the spleen (70 ml/m<sup>3</sup>) 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<sup>3</sup> 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<sup>3</sup>, 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<sup>3</sup> group

compared with the 280 ml/m<sup>3</sup> group (an about 8-fold concentration difference), but not in the 10 ml/m<sup>3</sup> group compared with the 70 ml/m<sup>3</sup> 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<sub>5</sub> and cytochrome P-450 levels and the activities of aminopyrine N-demethylase, dinemorphane N-demethylase and isoprene epoxidase were unchanged compared with controls (Del Monte et al. 1985).

## 6 Manifesto

After inhalation at 70 ml/m<sup>3</sup> 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<sup>3</sup>, no significantly increased tumour incidence was recorded. After isoprene inhalation exposure, at the lowest concentration tested of 220 ml/m<sup>3</sup> 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<sup>3</sup> 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<sup>3</sup> makes no significant contribution to the cancer risk. A MAK value of 3 ml/m<sup>3</sup> 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<sup>3</sup>. 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<sup>3</sup>, but not at 70 ml/m<sup>3</sup> (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<sup>3</sup>, 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<sup>3</sup> (highest tested concentration), and in mice 280 ml/m<sup>3</sup> (decreased foetal weight at

1400 ml/m<sup>3</sup> and above) (NTP 1995). The difference to the MAK value of 3 ml/m<sup>3</sup> 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](http://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  $\alpha$ ,  $\mu$ ,  $\mu$  and  $\theta$ -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 B6C3F<sub>1</sub> 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<sub>1</sub> 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, Holzknacht 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 Профессиональное Заболевания, 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<sub>1</sub> 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 Болез, Journal of Ear, Nose and Throat Diseases) *Zh Ushn Nos Gori Bolezn* 29: 79–83
- Mitsui T, Naitho K, Tsuda T, Hirabayashi 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 B6C3F<sub>1</sub> 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 oncogenic study of isoprene in B6C3F<sub>1</sub> 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 Piperlylen im akuten Versuch (Investigation of selective gonadotoxic activity of isoprene and piperlylene in an acute study) (German, translation from the Russian in: Гигиена Производственной и Окрашущей Среди, Охрана Здоровья Рабочих в Нефтехимический Пром-сти, Professional Hygiene in the Painting Branch and Petrochemical Industry) (deutsche Übersetzung aus dem Russischen). *Gigiena Proizvodstvennoj i Okruzasuscej 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 (+)-  $\alpha$ -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). *Azerb Med Zhyp 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- and 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 Spectrometry* 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, Papa-georgiou 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 [<sup>14</sup>C]butadiene or [<sup>14</sup>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 Spectrometry* 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

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