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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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Isoprene

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1 Summary and assessment

On inhalation administration of increasing concentrations of isoprene, the detectable percentages of dose in the body and the excrements decrease continuously. This means that as the dose increases, less and less of it is absorbed and/or increasing proportions of it are excreted without undergoing biotransformation (rat, on single inhalation: up to 95.5%, mouse: up to 97.7%). It has been demonstrated that there are great interspecies differences with respect to epoxide hydrolase activity, the lowest values being found in the mouse. These differences are very likely to be the underlying reason for the species-related differential toxicity of isoprene. In mice, steady-state blood levels are reached within 15 to 30 minutes of inhalation exposure. On inhalation of radioactively labelled isoprene by rats and mice, between 52 and 75% of metabolite-associated radioactivity is excreted in the urine and between 6 and 37% in the faeces, while 2 to 20% is eliminated in the exhaled air. Upon single intraperitoneal administration of ^{14}C-labelled isoprene to rats and mice, elimination of the administered radioactivity is as follows: 47 to 50% in the exhaled air, 29 to 33% in the urine, 0.3 to 7.2% in the faeces and 1.4 to 1.9% in the form of CO_2. In mice, unchanged isoprene accounts for 94% of the exhaled radioactivity. On inhalation of the radioactively labelled chemical by rats, unchanged isoprene as well as metabolite-associated radioactivity are predominantly found in the liver, blood and adipose tissue. In rats and mice, the highest tissue concentrations following intraperitoneal injection of ^{14}C-labelled isoprene are present in the kidneys and the urinary bladder. The metabolites formed in vivo have not yet been fully characterised. In more recent studies in rats and mice, the major urinary metabolites of isoprene found after intraperitoneal injection of ^{14}C-labelled isoprene have been identified as 2-hydroxy-2-methyl-3-butenoic acid, *trans*-3-methyl-2-butene-3,4-diol, the glucuronide conjugate of *trans*-3-methyl-1-butene-3,4-diol and a metabolite which was

not further characterised. In vitro the major metabolites to have been identified in microsomal preparations from mouse, rat, Syrian hamster, rabbit and human liver are 3,4-epoxy-3-methyl-1-butene and to a lesser extent 3,4-epoxy-2-methyl-1-butene, which are hydrolysed by the enzyme, epoxide hydrolase, to *trans*-3-methyl-1-butene-3,4-diol and *trans*-2-methyl-1-butene-3,4-diol, or oxidised by cytochrome P-450 to form the diepoxide, 2-methyl-1,2,3,4-diepoxybutane.

Isoprene has been shown to be of low toxicity following acute oral administration, dermal application and inhalation exposure (LD_{50} rat oral 2125 mg/kg body weight; LD_{50} rat dermal > 681 mg/kg body weight; LC_{50} rat (4 hours) > 101000 mg/m³ (males) and > 98000 mg/m³ (females); LC_{50} mouse (4 hours) > 31500 mg/m³). The LD_{50} value determined in rats after intraperitoneal administration is 1390 mg/kg body weight. The clinical signs of toxicity observed include sedation and impaired breathing. No cumulative effect is detectable after 5-day oral administration to rats. In the preliminary studies to subchronic toxicity studies in rats and mice conducted as part of the U.S. National Toxicology Program (NTP), inhalation by male and female rats of vaporous isoprene for 14 days did not result in any substance-related changes up to the highest test concentration of 7000 ppm (equivalent to 19530 mg/m³). Male mice subjected to 14-day inhalation of vaporous isoprene showed retarded body weight development and changes in organ weights (increase in liver weights, decreases in thymus, spleen and testicular weights) and haematology parameters (decreases in red blood cell count, haemoglobin concentration and erythrocyte mean corpuscular volume) at the highest concentration (7000 ppm). Microscopic findings in male mice exposed to 7000 ppm included thymic and testicular atrophy, and at levels of 1750 ppm (equivalent to 4883 mg/m³) or higher, olfactory epithelial degeneration in the nasal cavities was detected. At all test concentrations (438 to 7000 ppm), epithelial hyperplasia of the forestomach was seen in male and female mice alike.

Isoprene has a mildly irritating effect on the skin of the rabbit.

In the preliminary studies to the carcinogenicity studies conducted within the NTP framework, 13-week exposure of rats and mice to isoprene vapour at concentrations of 0 (controls), 70, 220, 700, 2200 and 7000 ppm (equivalent to 0, 195, 614, 1953, 6138 and 19530 mg/m³) did not lead to treatment-related changes in rats. The female mice exhibited impairment of

body weight gain at all concentrations. At levels of 220 ppm or higher, macrocytic anaemia was found in both sexes. Changes in organ weight were observed in the testes (decrease, from 2200 ppm), the liver (increase in male and female mice at 7000 ppm), the spleen (decrease, from 220 ppm in male mice and from 700 ppm in female mice) and the kidneys (increase, from 220 ppm in female mice). Microscopic examination revealed changes in the forestomach (epithelial hyperplasia, from 700 ppm), in the nasal cavity (olfactory epithelial degeneration in male mice at 7000 ppm), the liver (hepatocellular hypertrophy, from 2200 ppm) and the testes (seminiferous tubule atrophy at 7000 ppm). (The results on sperm motility and vaginal cytology are summarised below in the paragraph on reproductive toxicity.)

In the Salmonella/microsome assay, isoprene and its two metabolites, 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene, gave negative results, whereas the diepoxide, 2-methyl-1,2,3,4-diepoxybutane, tested positive. In CHO cells isoprene induces no increase in either the frequency of sister chromatid exchange or the incidence of chromosome aberrations, either in the absence or the presence of metabolic activation. In vivo, repeated inhalation administration of isoprene (12-day study) has been shown to cause a significant increase in the frequency of sister chromatid exchange in the bone marrow of mice. On repeated inhalation exposure of mice (12 days to 80 weeks) in several studies, increased counts of micronucleated erythrocytes have been observed in peripheral blood. Following repeated 12-day exposure to isoprene, no indications have been found of any potential to produce chromosome aberrations in the bone marrow of mice. In rats, no increased frequency of micronuclei in lung fibroblasts was detected after 4 weeks of inhalation administration. In amplified DNA from Harderian gland neoplasms of mice, an increase in the frequency of K-*ras* and H-*ras* proto-oncogene mutations has been observed. The mice were exposed to isoprene for 26 months. Hence, the chemical is genotoxic.

In the 26-week NTP study, exposure of male rats to isoprene at levels of 0 (controls), 70, 220, 700, 2200 and 7000 ppm (equivalent to 0, 195, 614, 1953, 6138 and 19530 mg/m³) resulted in significant, but reversible, changes in organ weight (liver at 7000 ppm and kidneys from 70 ppm). At the end of the 26-week exposure period an increased incidence of interstitial cell hyperplasia of the testes was observed at levels of 700 ppm and above, and after a 26-week post-exposure observation period the incidence of interstitial cell adenomas was increased at 700 ppm and higher.

In a 2-year study in rats exposed to isoprene concentrations of 0 (controls), 220, 700 and 7000 ppm (equivalent to 0, 614, 1953 and 19530 mg/m³), the male rats showed increased incidences of interstitial cell adenomas of the testes, of renal tubule adenomas and renal tubule hyperplasia, and of splenic fibrosis at 700 ppm and higher; at the top concentration, increased incidences of mammary gland fibroadenoma and of hyperplasia of the parathyroid gland were seen. In female rats all test concentrations, 220 ppm and higher, were associated with an increased incidence of mammary gland fibroadenomas. In the brains of the female rats a number of neoplasms, which were very rarely or never seen in historical controls, were diagnosed and assessed as being possibly related to treatment. In male mice, the 26-week exposure to isoprene levels of 0 (controls), 70, 220, 700, 2200 and 7000 ppm (equivalent to 0, 195, 614, 1953, 6138 and 19530 mg/m³) affected body weight gain at the highest concentration as well as causing abnormal posture and hindlimb paralysis, both of which were reversible. (A more detailed summary is given below in the paragraph on neurotoxicity studies.) Mortality was concentration-dependently increased from 2200 ppm. At levels of 700 ppm and above, macrocytic anaemia and changes in organ weights (increased liver weights, reversibly decreased testicular weights, irreversibly decreased brain weights) were observed. At the end of a 26-week observation period, the mice were observed to have significantly increased incidences of tumours of the liver (from 700 ppm), the lung (from 2200 ppm), the forestomach (at 7000 ppm) and the Harderian gland (from 700 ppm). In a further inhalation carcinogenicity study in female and male mice exposed to isoprene concentrations of 0 (controls), 10, 70, 140, 280, 700 2200 ppm (equivalent to 0, 28, 195, 391, 781, 1953 and 6138 mg/m³) for 20, 40 or 80 weeks, significant increases were seen in incidences of Harderian gland adenomas at 70 ppm and higher, hepatocellular adenomas at 140 ppm and higher, and primary alveolar adenomas and carcinomas at 700 ppm and higher. A concentration-independent increase in the incidence of histiocytic sarcoma was observed at a concentration of 280 ppm and higher. Slight, but not statistically significant, increases in the incidences of squamous cell carcinoma of the forestomach as well as of haemangiosarcomas in the spleen and heart have also been detected. Metaplasia of the olfactory epithelium has been observed at exposure levels of 70 ppm and above. In male rats and mice, isoprene has thus unequivocally been demonstrated to be a carcinogen. In female rats, there is evidence to suggest that isoprene has a carcinogenic potential.

In pregnant rats, exposure to isoprene on days 6 to 19 of gestation has not been found to cause maternal toxicity, foetotoxicity and teratogenicity up to the highest test concentration of 7000 ppm (equivalent to 19530 mg/m³). Mice exposed to isoprene on days 6 to 17 of gestation have shown toxicity in the mothers and foetuses from 1400 ppm and 280 ppm, respectively. Teratogenic effects have not been observed up to and including the highest test dose of 7000 ppm. In male mice, 13-week exposure (see 13-week NTP study) to 0 (controls), 70, 700 and 7000 ppm (equivalent to 0, 195, 1953 and 19530 mg/m³) resulted in decreased testicular and epididymal weights at 700 ppm and higher and caused atrophy of the seminiferous tubules at 7000 ppm. Sperm head count, sperm concentration as well as sperm motility were decreased at levels of 700 ppm and above. Female mice exposed to 7000 ppm exhibited a significant increase in oestrous cycle length. Following 30-day intraperitoneal administration to mice, isoprene (500 mg/kg body weight/day) proved to be toxic to the ovaries.

Following 26-week exposure (see NTP carcinogenicity study) of male mice to isoprene concentrations of 0 (controls), 70, 220, 700, 2200 and 7000 ppm (equivalent to 0, 195, 614, 1953, 6138 and 19530 mg/m³), forelimb and hindlimb grip strengths of animals exposed to levels of 220 ppm and above was significantly less than those of the control group, a finding which was reversible within the 26-week post-exposure observation period. Histopathology at the end of the exposure period revealed low-grade degenerative changes in the white matter of the spinal cord at concentrations of 2200 ppm and higher, and of the sciatic nerve at 7000 ppm. At the end of the observation period, all exposure groups exhibited a significantly increased incidence of spinal cord degeneration, and at 700 ppm and higher, relative brain weights were significantly decreased as compared with the control group.

In rats and mice, isoprene forms haemoglobin adducts following intraperitoneal and inhalation administration.

In humans, inhalation of isoprene causes mucous membrane irritation. The odour threshold concentration is given as 10 mg/m³. Numerous studies in humans have identified isoprene as a substance which is formed endogenously and subsequently eliminated in the exhaled air.

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (“MAK-Kommission”) of the Deut-

sche Forschungsgemeinschaft will investigate the possibility of establishing a MAK value for the chemical as well as the necessity for classification of its carcinogenic potential.

2 Name of substance

2.1	Usual name	Isoprene
2.2	IUPAC name	2-Methyl-1,3-butadiene
2.3	CAS No.	78-79-5
2.4	EINECS No.	201-143-3

3 Synonyms, common and trade names

1,3-Butadiene, 2-methyl-
Hemiterpene
Isopentadiene
Isopren
β-Methyl bivinyl
2-Methyl bivinyl
β-Methylbivinyl
Methylbutadien-1,3
2-Methylbutadiene
2-Methyl-1,3-butadiene
2-Methylbuta-1,3-diene
2-Methyldivinyl
2-Methylethene

4 Structural and molecular formulae

4.1 Structural formula

$$CH_2{=}\underset{\displaystyle CH_3}{\underset{|}{C}}{-}CH{=}CH_2$$

4.2 Molecular formula C_5H_8

5 Physical and chemical properties

5.1 Molecular mass, g/mol 68.12

5.2	Melting point, °C	–146	(EC, 1996)
		–145.9	(Lide and Frederikse, 1996)
		–145.96	(Weitz and Löser, 1989)
5.3	Boiling point, °C	34 (at 1013 hPa)	(EC, 1996)
		34.0 (at 1013 hPa)	(Lide and Frederikse, 1996)
		34.059 (at 1013 hPa)	(Weitz and Löser, 1989)
5.4	Vapour pressure, hPa	98 (at –20 °C)	(Weitz and Löser, 1989)
		133 (at –16 °C)	(EC, 1996)
		264 (at 0 °C)	(Lide and Frederikse, 1996; Weitz and Löser, 1989)
		405 (at 10 °C)	(Hoechst, 1995)
		533 (at 15.4 °C)	(EC, 1996)
		607 (at 20 °C)	(Weitz and Löser, 1989)
		618 (at 21 °C)	(Hoechst, 1995)
		734 (at 25 °C)	(Lide and Frederikse, 1996)
		793 (at 21 °C)	(EC, 1996)
		1013 (at 34.059 °C)	(Weitz and Löser, 1989)
		1238 (at 40 °C)	(Weitz and Löser, 1989)
		1621 (at 49 °C)	(Hoechst, 1995)
		2291 (at 60 °C)	(Weitz and Löser, 1989)
		3921 (at 80 °C)	(Weitz and Löser, 1989)
		6295 (at 100 °C)	(Weitz and Löser, 1989)
5.5	Density, g/cm³	0.681 (at 20 °C)	(EC, 1996)
		0.679 (at 20 °C)	(Lide and Frederikse, 1996)
		0.68095	(Weitz and Löser, 1989)
		0.69 (at 20 °C)	(Hoechst, 1995)
5.6	Solubility in water	0.3 g/l (at 20 °C)	(Shell, 1993; Hoechst, 1995)
		0.38 g/l (at 20 °C)	(EC, 1996)
		0.029 mol% (0.29 mol/l; at 20 °C)	(Weitz and Löser, 1989)

5.7	Solubility in organic solvents	Miscible in all proportions with ethanol, diethyl ether, acetone, benzene (Weitz and Löser, 1989; Lide and Frederikse, 1996)
5.8	Solubility in fat	Partition coefficient n-octanol/water log P_{ow}: 3.2 –4.5 (at 20 ° C; measured) (EC, 1996) log P_{ow}: 2.35 (Hoechst, 1995)
5.9	pH value	–
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 2.79 mg/m³ 1 mg/m³ ≙ 0.358 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

For the synthesis of poly(*cis*-1,4-isoprene) (isoprene rubber, IR; tyre production); for the manufacture of styrene-isoprene-styrene (SIS) block copolymers and butyl rubber (isobutene-isoprene rubber, IIR, a copolymerisate with isobutene); in the production of hydrocarbon resins (petroleum resins); for the synthesis of terpenes (Weitz and Löser, 1989).

7 Experimental results

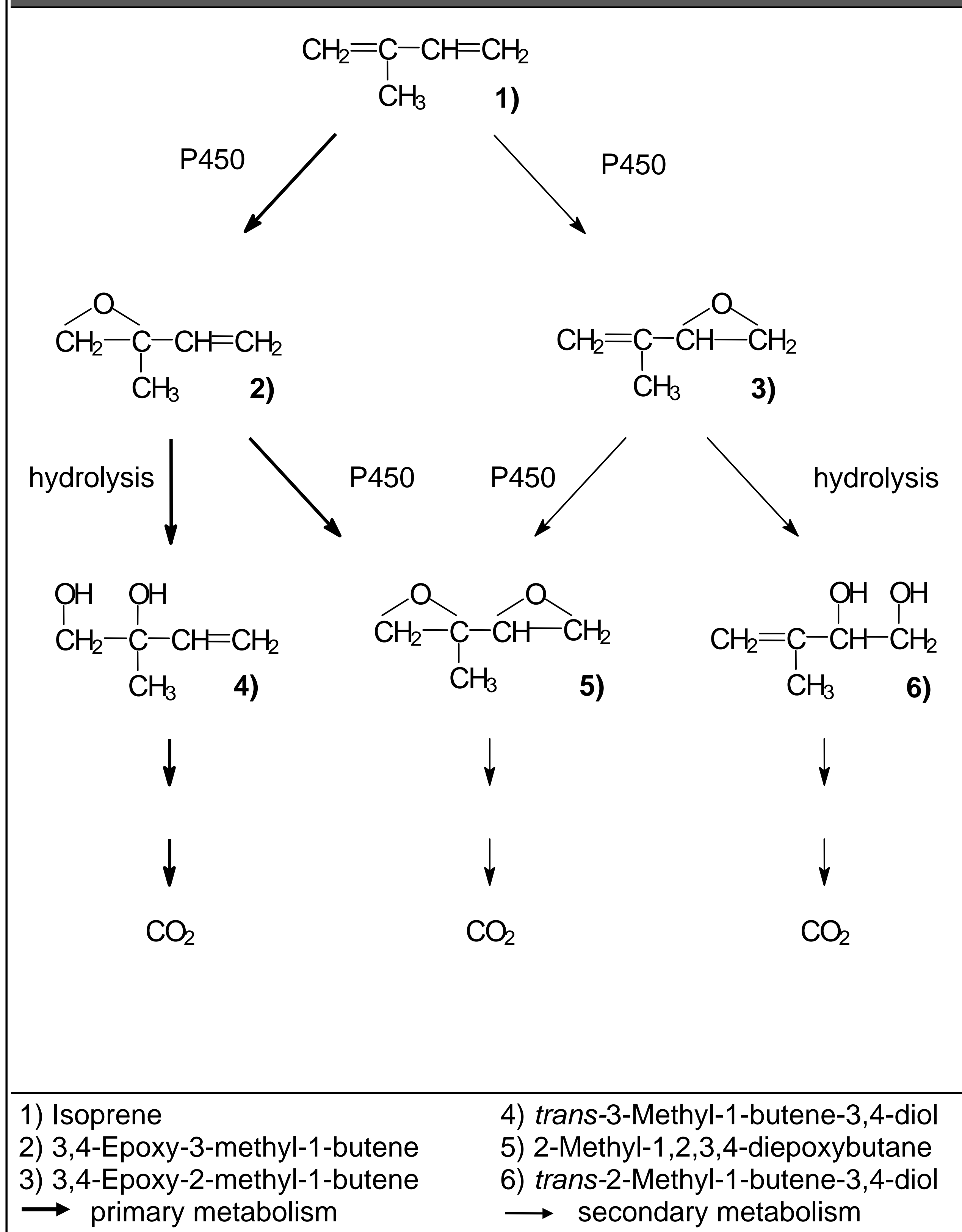
7.1 Toxicokinetics and metabolism

In vitro

Microsomal preparations from livers of male Swiss mice (given 100 mg phenobarbital/kg body weight intraperitoneally on 3 consecutive days or a single intraperitoneal injection of 80 mg 3-methylcholanthrene 48 hours before preparation), Wistar rats, Syrian hamsters and New Zealand rabbits (0.1% phenobarbital in the drinking water for 7 days) were incubated at 37 °C with 100 µl of a 0.5 M methanol solution of isoprene. The metabolites formed in the process were identified by gas chromatography. The microsomal mono-oxygenases metabolised isoprene to the corresponding monoepoxides (cf. Figure 1). The reaction was NADPH- and O_2-dependent

and was inhibited by cytochrome P-450 inhibitors, such as carbon monoxide, SKF 525-A and metapyrone. Only a few minutes after induction, 3,4-epoxy-3-methyl-1-butene was detectable as the major metabolite, which was then hydrolysed to *trans*-3-methyl-1-butene-3,4-diol. To a lesser extent, 3,4-epoxy-2-methyl-1-butene was formed (rat 25%, mouse 20%, hamster 17%, rabbit 14% of the amount of 3,4-epoxy-3-methyl-1-butene), which was subsequently hydrolysed to *trans*-2-methyl-1-butene-3,4-diol. Moreover, it was demonstrated that 3,4-epoxy-2-methyl-1-butene may be transformed into the mutagenic diepoxide, 2-methyl-1,2,3,4-diepoxybutane (cf. Section 7.6.1; Gervasi et al., 1985). The epoxidations followed Michaelis-Menten kinetics. In the mouse and rabbit, enzyme induction by phenobarbital or 3-methylcholanthrene had no effect on the kinetic parameters (Del Monte et al., 1985; Longo et al., 1985; Gervasi and Longo, 1990).

Figure 1. Biotransformation of isoprene



In addition, the metabolism of isoprene (purity: 99%) was studied in vitro by incubation with microsomes obtained from transfected cell lines (AHH-1 TK+/- human lymphoblastoid cell lines) expressing the human cytochrome P-450 enzymes 1A1, 1A2, 2A6, 2B6, 2C9, 2D6, 2E1 or 3A4 and showing no detectable epoxide hydrolase activity, and in microsomes from human, mouse and rat liver. The formation of the monoepoxides, 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene, and of the diepoxide, 2-methyl-1,2,3,4-diepoxybutane, was ascertained by gas chromatography. Incubations were performed for 15 minutes at 37 °C with 600 μM isoprene or monoepoxide and microsomal protein concentrations of approx. 1 mg/ml (representing V_{max} conditions). Cytochrome P-450 2E1 showed the highest activity towards the formation of 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene (259 and 30 pmol/minute x mg microsomal protein, respectively), followed by cytochrome P-450 2B6 (75 and < 14 μmol/minute x mg microsomal protein, respectively). The rate of formation of 3,4-epoxy-3-methyl-1-butene thus was 5 to 8 times higher than that of 3,4-epoxy-2-methyl-1-butene. Cytochrome P-450 2E1 was the only enzyme to exhibit detectable formation of the diepoxide of isoprene. Both monoepoxides were oxidised by cytochrome P-450 2E1 to the diepoxide at similar rates (178 and 107 pmol/minute x mg microsomal protein, respectively). To investigate the role of cytochrome P-450 2E1 in hepatic metabolism, isoprene and the two monoepoxides were incubated with microsomes prepared from human liver. In the human liver microsomes, the rate of formation of 3,4-epoxy-3-methyl-1-butene was approx. 4 times higher than that of 3,4-epoxy-2-methyl-1-butene. Both monoepoxides were found to have similar reactions rates with regard to 2-methyl-1,2,3,4-diepoxybutane formation. The rates of monoepoxide formation and of diepoxide formation were correlated in a statistically significant manner with the activity of cytochrome P-450 2E1. In the presence of an epoxide hydrolase inhibitor (cyclohexene oxide), analysis showed that the monoepoxide amounts in mouse (CD-1, B6C3F1), rat (Wistar) and human liver microsomes were very similar, with 3,4-epoxy-3-methyl-1-butene amounts being 2.2 (rat) to 3.3 (humans) times higher than those of 3,4-epoxy-2-methyl-1-butene. In the absence of epoxide hydrolase inhibitor, striking differences were observed between the various species. While the monoepoxide amounts detected in mouse liver microsomes in the presence and absence of the inhibitor were similar, rat and, in particular, human liver microsomes showed much lower amounts of 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-

methyl-1-butene (approx. 50 and 4%, and approx. 70 and 25% of the respective monoepoxide detected in the presence of inhibitor). The monoepoxide amounts detected in mouse liver microsomes were twice as high as in rat liver microsomes and 15 times higher than in human liver microsomes. In the incubations with mouse, rat and human liver microsomes, similar amounts of diepoxide were formed from 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene (CD-1 mouse 1210 and 886, B6C3F1 mouse 806 and 967, Wistar rat 1150 and 1360, humans 780 and 666 pmol/minute × nmol P-450, respectively). This study was able to demonstrate that 3,4-epoxy-3-methyl-1-butene can also undergo further oxidation to the diepoxide. Moreover, the authors were able to show that there are great differences between species with regard to the enzyme, epoxide hydrolase, which catalyses hydrolysis of the epoxides. It is highly likely that this difference in expoxide hydrolase activity is responsible for the species-dependent differential toxicity of isoprene. The authors' conclusion is that mice lack sufficient epoxide hydrolase to remove the monoepoxides rapidly (Bogaards et al., 1996, 1997).

In-vitro studies investigating the stereoselectivity of isoprene metabolism in liver microsomes from male F344 rats and B6C3F1 mice have confirmed the metabolic pathway diagram presented above. In these studies, isoprene was epoxidised by cytochrome P-450 to the isomeric monoepoxides, 3,4-epoxy-2-methyl-1-butene and 3,4-epoxy-3-methyl-1-butene, exhibiting slight, but different product enantioselectivity. Only in the studies with mouse liver microsomes was clear regioselectivity observed. Both monoepoxides were further epoxidised to 2-methyl-1,2,3,4-diepoxybutane, the reaction showing substrate enantioselectivity, product diastereoselectivity and product enantioselectivity. The epoxide hydrolase-catalysed hydrolysis by rat and mouse liver microsomes exhibited substrate enantioselectivity. Whilst (R)-3,4-epoxy-2-methyl-1-butene was conjugated preferentially with glutathione, a reaction catalysed by glutathione S-transferase, no enantiomer differentiation took place in the case of 3,4-epoxy-3-methyl-1-butene conjugation (Wistuba et al., 1994).

In a further study of the stereochemistry of isoprene metabolism, liver microsomes obtained from different species (male and female Sprague-Dawley rats, male Wistar rats, male B6C3F1 mice, male New Zealand rabbits, female Beagle dogs, monkeys, male and female human donors) were incubated with 0.30 mmol isoprene (equivalent to 20 mg) for 30 minutes at

37 °C. In order to obtain elevated levels of cytochrome P-450 2E1, 1A1 or 2B, liver microsomes from male Wistar rats induced with pyrazole, β-naphthoflavone or phenobarbital were also used. Control incubations were carried out in the absence NADPH (nicotinamide-adenine dinucleotide phosphate, reduced form), in the presence of heat-inactivated liver microsomes, of microsomes and in the absence of test substance. The addition of 1,1,1-trichloropropylene oxide was used to inhibit enzymic hydroysis of the monoepoxides by epoxide hydrolase. On incubation, the formation of monoepoxide isomers was monitored by means of head space gas chromatographic analysis. In all microsomal incubations the isomer, 3,4-epoxy-3-methyl-1-butene, was the major monoepoxide metabolite. On account of low the compound's volatility, detection of 2-methyl-1,2,3,4-diepoxybutane was not possible by means of the head-space technique. Analysis of the monoepoxides formed by liver microsomes from male Wistar rats demonstrated that the S-enantiomer of 3,4-epoxy-3-methyl-1-butene was preferentially formed with an R : S-enantiomer ratio of 45 : 55. In the other incubations, technical obstacles prevented a more accurate determination of the 3,4-epoxy-3-methyl-1-butene enantiomer ratio. The largest amount of 3,4-epoxy-2-methyl-1-butene was formed in the liver microsomes from male Wistar rats (cf. Table 1). The ratios of the R- and S-enantiomers of 3,4-epoxy-2-methyl-1-butene found in the various species are presented in Table 1.

Beginning of Table 1

Table 1. Ratios (R : S) and amounts (R + S) of 3,4-epoxy-2-methyl-1-butene found in incubations with liver microsomes from different species

Species (strain, sex)	Ratio (R : S)	Relative amount (R + S)
Rat (Wistar, male)	33 : 67 (n = 6)	100*
Rat (Wistar, male, cytochrome P-450 2E1-induced)	35 : 65 (n = 2)	64
Rat (Wistar, male, cytochrome P-450 2B-induced)	40 : 60 (n = 2)	86
Rat (Wistar, male, cytochrome P-450 1A1-induced)	33 : 67 (n = 2)	36
Rat (Sprague-Dawley, female)	50 : 50 (n = 4)	18
Rat (Sprague-Dawley, male)	36 : 64 (n = 4)	58
Mouse (B6C3F1, male)	50 : 50 (n = 3)	67

Table 1. Ratios (R : S) and amounts (R + S) of 3,4-epoxy-2-methyl-1-butene found in incubations with liver microsomes from different species		
Species (strain, sex)	Ratio (R : S)	Relative amount (R + S)
Rabbit (New Zealand, male)	50 : 50 (n = 4)	37
Dog (beagle, female)	57 : 43 (n = 4)	76
Monkey (male)	57 : 43 (n = 4)	46
Man (male, one donor)	55 : 45 (n = 2)	19
Man (male, 7 donors, pooled)	54 : 46 (n = 2)	38
Man (female, 7 donors, pooled)	50 : 50 (n = 2)	27
* Corresponds to 4.2 nmol/mg protein/minute		

End of Table 1

The ratios of the R- and S-enantiomers of 3,4-epoxy-2-methyl-1-butene produced by liver microsomes from rat and mouse varied significantly, as did the ratios found for the two rat sexes. Liver microsomes from B6C3F1 mice and from female Sprague-Dawley rats thus showed no stereochemical preference, whilst in the case of male Sprague-Dawley rats an R : S ratio of 36 : 64 (cf. Table 1) indicated a preference for the S-enantiomer. Similar ratios were found for the microsomes from male Wistar rats. Induction of the various cytochrome P-450 isoenzymes had no effect on enantiomer ratio. Rabbits did not display preferential formation of either one of the enantiomers, either. In the dog and monkey, there was a slight predominance of R-enantiomer (57 : 43). Similar results were also seen in the experiments carried out using human liver microsomes. According to the authors, the significance of these findings for the toxicology of isoprene is not known (Small et al., 1997).

The partition coefficients were determined for isoprene (purity: > 98%) with physiological saline solution, olive oil, blood and homogenates of fat, liver and muscle tissue from male Sprague-Dawley rats (weighing 200 to 300 g), and air, employing a head space gas chromatographic method. The initial concentrations of the test chemical at the beginning of the experiments ranged from approx. 200 to 400 ppm (no further details). Incubation of the vials was carried out at 37 °C for 1 hour or 3 hours (no further details). The following partition coefficients were determined: physiological saline solution : air 0.21 ± 0.02; olive oil : air 8.81 ± 0.15; blood : air 1.87 ± 0.10; fat : air 72.0 ± 2.4; liver : air 3.12 ± 0.87; muscle : air 2.04 ± 0.27 (Gargas et al., 1989).

In addition, the partition coefficients of 99% pure isoprene were ascertained with blood obtained from mice, rats and humans and tissue samples from male Sprague-Dawley rats (fat, muscle, liver, kidney) at 37 °C with the aid of the head space technique. The blood : air partition coefficients were found to be 2.4 ± 0.47 (n = 6), 2.33 ± 0,64 (n = 5) and 0.75 ± 0.08 (n = 8) in mice, rats and humans, respectively. The following partition coefficients were determined for the rat tissues: fat : air 61.5 ± 15.4 (n = 3), muscle : air 1.48 ± 0.05 (n = 3), liver : air 1.93 ± 0.79 (n = 3) and kidney : air 1.07 ± 2.43 (n = 2). In the authors' opinion, the difference in the blood : air partition coefficients between rodents and humans might result from the differences in affinity of blood proteins towards volatile lipophilic organic compounds (Filser et al., 1996). No statement was made regarding the effect that blood lipids might have.

In vivo

Groups of 30 male F344 rats (11 to 13 weeks of age and weighing 190 to 250 g) underwent head-nose exposure to ^{14}C-labelled isoprene (purity: > 99%) at concentrations of 8, 266, 1480 and 8200 ppm (equivalent to 22, 742, 4129 and 22878 mg/m³) for 6 hours (only 5.5 hours at the 8200 ppm level). Per group, 5 animals were placed in plethysmographic exposure tubes and their respiratory patterns measured as minute volume, tidal volume and breathing frequency. After cessation of exposure, the animals were killed and the content of ^{14}C-label in their bodies was determined. In 4 animals/group the urine and faeces were quantitated for ^{14}C at 9, 18, 24, 30, 42, 54 and 66 hours after the end of exposure, while the amount ^{14}C in the exhaled air was measured at 9, 18, 36 and 54 hours after exposure. At the end of the 66-hour post-exposure observation period, the amount of radioactivity remaining in the body was quantified. During the exposure period, 3 animals out of each group were sacrificed after 20, 120 or 360 minutes (330 minutes for the 8200 ppm exposure) and the blood samples analysed for metabolites formed in the process by means of vacuum line-cryogenic distillation. This procedure permits separation of compounds by fractionation according to their boiling points. The metabolites in the tissues (nose, lung, liver, kidneys, subcutaneous fatty tissue, blood) of 4 animals from each group exposed to 1480 ppm were determined at 20 minutes, 2 hours and 6 hours after commencement of exposure as well as after an 18-

hour recovery period, again by vacuum line-cryogenic distillation. The percentage of inhaled ^{14}C that was retained in the form of metabolites at the end of the 6-hour exposure dropped with increasing isoprene concentration. Up to 95.5% of the radioactivity was not absorbed by inhalation and/or excreted as unchanged isoprene (cf. Table 2).

Table 2. Retention of ^{14}C-labelled isoprene following inhalation by rats (5 animals/group)

Isoprene concentration (ppm)	Exposure duration (hours)	Specific activity (nCi/µmol)	Minute volume (l)	Total isoprene inhaled (µmol)	Total ^{14}C retained at cessation of exposure (µmol)	Retained ^{14}C/ inhaled ^{14}C (%)
8	6	254	0.185 ± 0.044	18	3.5 ± 0.4	19.0 ± 3
266	6	10.4	0.230 ± 0.052	736	67.1 ± 8.1	9.1 ± 0.8
1480	6	11.5	0.207 ± 0.065	3650	211.0 ± 12	5.8 ± 0.9
8200	5.5	1.6	0.157 ± 0.013	14200	641.0 ± 61	4.5 ± 0.7

At the lower concentrations, a higher percentage of inhaled isoprene underwent metabolism compared with the higher concentrations (8 ppm: 25.3 ± 0.7%; 266 ppm: 12.0 ± 1.1%; 1480 ppm: 4.7 ± 0.8%; 8200 ppm: 3.6 ± 0.7%; cf. Table 3). In all concentration groups, more than 75% of the metabolite-associated radioactivity was excreted in the urine within 66 hours. The half-time of urinary excretion was 10.2 ± 1 hour. Faeces and exhaled $^{14}CO_2$ together accounted for between 3 and 20% of ^{14}C excretion. A significant increase in faecal elimination of ^{14}C occurred in the 8200 ppm groups (cf. Table 3).

Table 3. Total excretion of isoprene metabolites in the rat 66 hours after exposure

Isoprene concentration (ppm)	Urine		Faeces		CO_2		Body	
	% Inhaled isoprene	% Total metabolites	% Inhaled isoprene	% Total metabolites	% Inhaled isoprene	% Total metabolites	% Inhaled isoprene	% Total metabolites
8	19.4	76.7	0.6	2.4	0.7	2.8	4.6	18.2
266	9.4	78.3	0.4	3.3	0.4	3.3	1.8	15.0
1480	3.8	81.3	0.08	1.7	0.09	1.9	0.8	16.6
8200	2.7	73.3	0.5	13.4*	0.2	5.3	0.3	8.0*

* Significantly different from the 8 ppm groups

The quantities of metabolites found in the blood increased with the duration of exposure and with increasingly higher concentrations (cf. Table 4).

Table 4. ^{14}C-Labelled isoprene and ^{14}C-labelled isoprene metabolites in the blood of rats (3 animals/group) upon inhalation administration					
Duration of exposure* (minutes)	Tentatively characterised metabolites	% of total activity in blood			
		8 ppm	266 ppm	1480 ppm	8200 ppm
20	Conjugates, tetrols	98.7	96.7	91.7	95.6
	diepoxides, diols	0.8	2.5	5.5	1.7
	monoepoxides	n.d.	n.d.	n.d.	0.5
	unchanged isoprene	n.d.	n.d.	1.8	0.7
	CO_2	0.5	0.8	0.9	1.5
120	Conjugates, tetrols	98.8	97.3	97.2	95.9
	diepoxides, diols	1.0	2.4	2.4	0.9
	monoepoxides	n.d.	n.d.	0.1	0.1
	unchanged isoprene	n.d.	n.d.	n.d.	3.7
	CO_2	0.2	0.3	0.3	0.3
360	Conjugates, tetrols	93.7	94.8	92.7	86.8
	diepoxides, diols	5.8	4.7	3.8	4.2
	monoepoxides	n.d.	n.d.	0.1	1.1
	unchanged isoprene	n.d.	n.d.	0.1	6.3
	CO_2	0.5	0.5	3.3	1.6
n.d. not detected		* At 8200 ppm, 330 minutes			

The highest percentage of radioactivity was associated with conjugates of isoprene metabolites (no further specification). Epoxides and free isoprene were detected only at the highest exposure levels. The distribution of ^{14}C-labelled isoprene and ^{14}C-labelled isoprene metabolites found in the examined tissues at the end of exposure is shown in Table 5.

Table 5. Distribution of ^{14}C-labelled isoprene and isoprene metabolites in the tissue of rats (4 animals/group) during and after inhalation of 1480 ppm ^{14}C-labelled isoprene						
Tentative identity of metabolites	nmol in total tissue					
	Nose	Lung	Liver	Kidneys	Fat tissue	Blood
After 20 minutes of exposure						
Conjugates, tetrols	72.5 ± 6.8	26.5 ± 1.7	493 ± 23	107 ± 1	293 ± 121	379 ± 55
Diepoxides, diols	45.5 ± 25.8	30.9 ± 6.0	49.9 ± 20.8	3.29 ± 2.04	466 ± 231	22.8 ± 3.0
Monoepoxides	1.45 ± 0.51	0.53 ± 0.18	0.39 ± 0.18	0.68 ± 0.46	67.8 ± 15.4	0.20 ± 0.20
Unchanged isoprene	n.d.	6.78 ± 6.74	1.94 ± 0.15	22.4 ± 10.0	193 ± 136	7.30 ± 2.70
CO_2	2.0 ± 0.38	1.42 ± 0.12	1.87 ± 0.66	0.47 ± 0.25	481 ± 381	3.70 ± 1.0
After 2 hours of exposure						
Conjugates, tetrols	360 ± 14	228 ± 14	3410 ± 210	666 ± 46	4500 ± 1200	1520 ± 65
Diepoxides, diols	12.2 ± 2.7	13.7 ± 1.6	82.7 ± 10.9	41.6 ± 16.3	823 ± 327	37.2 ± 14.1
Monoepoxides	0.97 ± 0.58	0.50 ± 0.39	2.24 ± 0.03	0.45 ± 0.27	89.7 ± 54	1.50 ± 0.30
Unchanged isoprene	n.d.	n.d.	n.d.	4.60 ± 4.60	51.2 ± 51.2	0.20 ± 0.20
CO_2	3.12 ± 0.91	0.70 ± 0.55	3.72 ± 0.97	2.45 ± 2.45	29.6 ± 14.6	4.20 ± 0.40
After 6 hours of exposure						
Conjugates, tetrols	737 ± 62	391 ± 17	5520 ± 125	1500 ± 50	37700±4120	5590 ± 470
Diepoxides, diols	27.6 ± 15.7	27.1 ± 13.2	235 ± 47	64.7 ± 15.9	2890 ± 1750	230 ± 96
Monoepoxides	3.99 ± 1.53	0.24 ± 0	0.50 ± 0.15	4.55 ± 2.53	72.4 ± 41.2	3.90 ± 0.60
Unchanged isoprene	44.5 ± 5.5	n.d.	0.08 ± 0.03	9.91 ± 5.52	556 ± 458	5.10 ± 1.80
CO_2	2.77 ± 0.62	1.07 ± 0.23	15.6 ± 1.4	12.6 ± 7.9	597 ± 392	201 ± 183
After 6 hours of exposure and 18 hours of recovery						
Conjugates, tetrols	171 ± 49	105 ± 18	870 ± 86	337 ± 41	2250 ± 150	464 ± 145
Diepoxides, diols	6.30 ± 3.80	1.20 ± 0.59	7.19 ± 2.88	3.26 ± 0.45	201 ± 132	1.67 ± 0.92
Monoepoxides	0.58 ± 0.19	0.43 ± 0.43	1.62 ± 0.45	0.42 ± 0.08	9.62 ± 3.08	0.43 ± 0.44
Unchanged isoprene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CO_2	2.13 ± 0.09	2.42 ± 0.80	1.86 ± 0.01	1.36 ± 0.33	54.3 ± 0.4	1.51 ± 1.35
n.d. not detected						

The liver, blood and, in particular, fatty tissue contained the highest levels of radioactivity at all time points investigated during exposure. In all tissues analysed, metabolite levels had dropped 18 hours after cessation of exposure and free isoprene was no longer detected in any of the tissues (Dahl et al., 1986, 1987, 1990).

Male B6C3F1 mice (approx. 28 g, 12 to 13 weeks old) were exposed to 0 (controls), 20, 200 and 2000 ppm isoprene (equivalent to 0, 46, 460 and 4600 mg/m³) for 6 hours. In 3 animals per exposure level, the blood levels of isoprene were ascertained by head-space gas chromatography 15, 30, 45 and 60 minutes as well as 2, 4 and 6 hours after the start of exposure. The steady-state blood levels were reached rapidly within 15 to 30 minutes. The isoprene levels measured in blood at up to 30 minutes were close to the limit of detection (approx. 15 ng/ml blood) in those animals exposed to 20 ppm. The mean steady-state blood levels at 30 minutes of exposure and up to 6 hours of exposure were 24.8 ± 3, 830 ± 51 and 6800 ± 400 ng isoprene/ml blood at the 20, 200 and 2000 ppm exposure levels, respectively, and thus they indicated a near proportional increase with increased exposure concentrations at the two highest exposure levels (Bond et al., 1991).

Per exposure concentration, 4 male B6C3F1 mice (12 to 13 weeks old, weighing approx. 28 g) were exposed (nose-only) to nominal concentrations of 20, 200 2000 ppm ^{14}C-labelled isoprene (equivalent to 46, 460 and 4600 mg/m³, respectively) for 6 hours. Immediately after cessation of exposure the animals were placed in metabolism cages for the collection of excreta, and urine and faeces were analysed for radioactivity after 3, 6, 9, 18, 24, 36, 42, 54 and 64 hours. The radioactivity in the expired air was measured after 1, 2, 3, 6, 9, 18, 24, 36, 42, 54 and 64 hours. The results are shown in Table 6. It was assumed that all of the ^{14}C was present in the form of metabolites.

Table 6. Excretion of isoprene metabolites in B6C3F1 mice over a period of 64 hours

	Isoprene		
	20 ppm	200 ppm	2000 ppm
Urine			
% of inhaled isoprene	2.5	4.8	1.7
% of total metabolites	52.4	56.3	73.4
Faeces			
% of inhaled isoprene	1.6	1.2	0.13
% of total metabolites	36.6	19.3	5.5
CO_2			
% of inhaled isoprene	0.1	1.2	0.4
% of total metabolites	2.1	18.4	18.0
Residue in body			
% of inhaled isoprene	0.4	0.3	0.07
% of total metabolites	8.9	6.0	3.1

Depending on the exposure concentration, from 52% (20 ppm isoprene) to 73% (2000 ppm isoprene) of the metabolite-associated radioactivity was excreted in the urine over a period of 64 hours. At the two lower concentrations of isoprene, 20 ppm and 200 ppm, 37% and 19% were excreted in faeces, respectively. Exhalation of $^{14}CO_2$ at the end of exposure ranged from 2% (20 ppm isoprene) to 18% (200 ppm and 2000 ppm isoprene). The predominant portion of the inhaled isoprene was not absorbed and/or was excreted unchanged (Bond et al., 1991).

Per exposure level, 4 male Sprague-Dawley rats and 4 male B6C3F1 mice each were given a single intraperitoneal administration of 10 µmol ^{14}C-labelled isoprene/kg body weight. Immediately after injection, the animals were placed in metabolism cages for 24 hours, and the exhalation of ^{14}C and the excretion of ^{14}C in the urine determined. In addition, the formation of haemoglobin adducts was calculated as a percentage of the administered dose under the assumption that total blood volume was 6% of body weight in both mice and rats. The results are presented in Table 7 (Sun et al., 1989).

Table 7. Excretion of ^{14}C in mice and rats within 24 hours of intraperitoneal injection of 10 µmol ^{14}C-labelled isoprene per kilogram body weight (Sun et al., 1989)

	Percentage of administered dose			
	Exhaled CO_2	Exhaled isoprene	Urine	Haemoglobin adducts
Mice	10 ± 0.4	46 ± 8	43 ± 0.3	0.08 ± 0.02
Rats	3 ± 0.1	19 ± 4	52 ± 5	0.06 ± 0.02

Male F344 rats investigated in 3 studies (Studies 1, 2 and 3 with 3, 3 and 5 animals/group, respectively) each received a single intraperitoneal dose of 66.6 mg, 66.2 mg and 66.4 mg, respectively, of ^{14}C-labelled isoprene/kg body weight (equivalent to 26.5, 27.2 and 15.1 µCi, respectively), formulated in maize germ oil (corn oil). After 3, 6, 12, 24 and 48 hours (Study 1), 6 and 24 hours (Study 2) and 6, 12 and 24 hours (Study 3), urine and faeces were collected and the levels of radioactivity measured. The exhaled air was analysed for radioactivity at 6, 12, 24 and 48 hours (Study 1), at 3, 6 and 24 hours (Study 2) and at 6, 12 and 24 hours (Study 3). At 24 hours (Studies 2 and 3) and 48 hours (Study 1) after administration, radioactivity

levels were measured in the blood, liver, lung, kidneys, adipose and muscle tissue, skin, brain, heart, testes and urinary bladder as well as the carcass. Approximately 50% of the administered radioactivity was exhaled in the form of volatile compounds. Urinary excretion, CO_2 and faecal excretion accounted for 29%, 1.4% and 0.3% of the administered radioactivity, respectively. Overall, excretion was rapid, with 51% being eliminated within 6 hours and 29% within a period of between 6 and 24 hours after administration. Only 3% of the administered radioactivity was recovered from the carcass and the tissue samples. Consistent with the predominantly urinary excretion of radiolabel, the kidneys were found to contain the highest levels of radioactivity (6.5 µg eq/g), followed by the urinary bladder (3.5 µg eq/g). The concentrations found in the remaining tissues were similar, ranging between 1 µg eq/g (adipose tissue and brain) and 2.8 µg eq/g (blood and liver). Analysis of the urine samples by high performance liquid chromatography (HPLC) revealed that no unchanged isoprene was excreted. At all time points of collection, 4 major metabolites of higher polarity than isoprene were detected. Isoprene was chiefly excreted as 2-hydroxy-2-methyl-3-butenenoic acid, *trans*-3-methyl-1-butene-3,4-diol and the glucuronide conjugate of *trans*-3-methyl-1-butene-3,4-diol. Structural identification of the fourth metabolite was not possible, but the compound was found not to contain any mercapturic acid derivatives. The fraction of this metabolite, 7 to 8% of total radioactivity, remained relatively unchanged over the entire collection period. The concentration of 2-hydroxy-2-methyl-3-butenenoic acid showed an increase over the whole collection period from approx. 28% to 63%, while the concentration of *trans*-3-methyl-1-butene-3,4-diol and that of the glucuronide conjugate of *trans*-3-methyl-1-butene-3,4-diol dropped from approx. 48% to 15% and from approx. 21% to approx. 9%, respectively (NTP, 1994).

A similar study was also carried out in male B6C3F1 mice (4 animals per group). The mice received a single intraperitoneal injection of 68.4 mg ^{14}C-labelled isoprene/kg body weight (equivalent to 10.2 µCi). Urine, faeces and exhaled air were collected after 6, 12 and 24 hours and analysed for radioactivity. The amounts of radioactivity in the tissues and the carcass were measured after 24 hours. As in the rat studies, the largest fraction of radioactivity (approx. 47%) was exhaled in the form of volatile compounds. Analysis by HPLC revealed that 94% of the exhaled radioactivity represented unchanged ^{14}C-labelled isoprene. Excretion in the urine, the faeces

and in the form of CO_2 accounted for approx. 33%, 7.2% and 1.9% of the administered radioactivity, respectively. Similar to the rat studies, excretion was rapid, with 50% of the radiolabel being eliminated within a period 6 hours and another 39% within a period of 6 to 24 hours after administration. At 24 hours after administration, 2% of the administered radioactivity was recovered from the carcass and the tissues. Again, the highest tissue concentrations were detected in the kidneys and the urinary bladder (2.2 µg eq/g). The concentration levels in the remaining tissues ranged from 0.5 (adipose tissue) to 1.8 or 2.0 µg eq/g (liver and testes). As in the rats, no unchanged isoprene was found in the urine. At all time points of collection, 4 major metabolites were detected. Structural identification of one of the metabolites was not possible, but the compound was found not to contain any mercapturic acid derivatives (see above). The concentration of this particular metabolite remained relatively constant (approx. 7%) over the whole 24-hour collection period. Further metabolites were detected including 2-hydroxy-2-methyl-3-butenenoic acid, the levels of which rose over the entire collection period (from approx. 27 to 63%), as well as *trans*-3-methyl-1-butene-3,4-diol and the glucuronide conjugate of *trans*-3-methyl-1-butene-3,4-diol, both exhibiting a decrease in concentration (from approx. 47 to 15% and approx. 21 to 9%, respectively) over the collection period. In the urine samples from the mice, low concentrations of additional metabolites were detected, though it was not possible to identify the structures of these compounds (NTP, 1994).

Male Wistar rats and B6C3F1 mice in groups of 2 and 5 animals, respectively, were placed in a closed system and exposed to initial isoprene concentrations ranging from 5 to 4000 ppm (14 to 11160 mg/m³) for up to 10 hours. The time course of the decline in isoprene concentration was monitored by gas chromatography. Below concentration levels of approx. 300 ppm (equivalent to approx. 840 mg/m³), the metabolic elimination rate in both species was directly proportional to the atmospheric concentration of isoprene. At higher exposure levels, saturation kinetics became apparent, with metabolic elimination rates reaching estimated maximum values of 130 µmol/(hour × kg) in rats at 1500 ppm (equivalent to 4185 mg/m³) and higher, and of 400 µmol/(hour × kg) in mice at 2000 ppm (equivalent to 5580 mg/m³) and higher. A comparison of clearance of uptake and metabolisation revealed that in rats approx. 15% was exhaled as unchanged isoprene at the 250 ppm level (equivalent to 698 mg/m³), and in mice ap-

prox. 25% was exhaled as parent compound at 300 ppm (equivalent to 840 mg/m^3). At higher concentration levels, the percentage of unchanged isoprene eliminated in the exhaled air increased in a concentration-dependent manner. At high levels, when metabolising enzymes were saturated, accumulation in the body was determined by the rates of inhalation and exhalation as well as the partition coefficient ("thermodynamic partition coefficient"). For the rat and the mouse, partition coefficients (body/air) were calculated to be 7.8 ± 3 and 7.0 ± 2, respectively. The half-lives of saturation in rats and mice were found to be 6.8 minutes and 4.4 minutes, respectively. In addition, the amount of endogenously produced isoprene was measured in untreated animals kept in a closed exposure system. The amounts calculated for rats and mice were 1.9 µmol/ (hour x kg) and 0.4 µmol/(hour x kg), respectively. A smaller fraction of the endogenously produced isoprene was exhaled by the animals as the parent compound, but a larger fraction was metabolised. The metabolic rate for isoprene in the rat and the mouse was calculated as 1.6 µmol/(hour x kg) and 0.3 µmol/(hour x kg), respectively (Peter et al., 1987, 1990).

The in vivo toxicokinetic data on isoprene exposure in rats and mice published by Peter et al. (1987, 1990), together with unpublished data from studies in rats and mice pretreated with dithiocarb (200 mg/kg body weight) (no further details), were reanalysed by means of a two-compartment model developed by Filser et al. (1996). According to this analysis, the maximum rates of metabolism in rats and mice were 26.5 and 10.2 µmol/hour/animal, respectively, and the values of the apparent Michaelis constant (K_{mapp} = hypothetical average isoprene concentration in the animal body at a rate of metabolism of $V_{max}/2$) were 0.026 and 0.060 mmol/l, respectively. These parameters were extrapolated from a two-compartment model to a physiologically based toxicokinetic model consisting of the compartments air, lung, richly perfused tissue (e. g. brain, intestines, kidney, spleen), fat, muscle and liver. From published data on cytochrome P-450 contents and activities, it was estimated that about 10% of the total activity of mixed-function mono-oxygenases is located outside the liver. Therefore, the maximum rate of metabolism obtained from the two-compartment model was split into V_{maxmo1} (90% in liver) and V_{maxmo2} (10% in richly perfused tissues). The value of the Michaelis constant K_{mmo} was assumed to be the same in venous blood of the liver and in richly perfused tissues. The newly calculated parameters of isoprene metabolism thus ob-

tained for the rat (body weight 250 g) and mouse (body weight 25 g) are summarised in Table 8.

Table 8. Metabolic parameters of isoprene in the rat and mouse according to a physiologically based toxicokinetic model		
	Rat	Mouse
V_{maxmo1} (µmol/hour/animal)	24.8	9.18
V_{maxmo2} (µmol/hour/animal)	2.75	1.02
K_{mmo} (mmol/l)	0.002	0.004

The chromatography method employed by Peter et al. (1987) in animals in the absence of exposure did not permit the distinction between endogenously produced acetone and isoprene. By an improved method, far smaller amounts (in the order of a few ppb; no further details) of endogenously formed isoprene were detected in Wistar rats. The data obtained with animals which were not exposed to isoprene were, therefore, not reanalysed (Filser et al., 1996).

In an inadequately documented study investigating the distribution of isoprene in rat tissues, 4-hour inhalation of about 180000 mg isoprene/m³ (a concentration which approximately corresponded to the LC_{50} value found in this study) resulted in the tissue concentrations summarised in Table 9.

Table 9. Distribution of isoprene in rat tissues upon inhalation	
Organ/Tissue	Isoprene concentration (mg/100 g tissue)
Brain	39.5 (n = 10)
Liver	43.3 (n = 10)
Kidney	39.6 (n = 7)
Spleen	28.0 (n = 7)
Kidney fat	257.7 (n = 7)
Subcutaneous fat	178.4 (n = 7)

According to the authors, there was a correlation between the isoprene concentration measured in brain and the narcotic effect (no further details; Shugaev, 1968, 1969).

Anaesthetised mongrel dogs of both sexes (weighing from 8 to 22 kg) inhaled isoprene vapours by means of a mask or an endotracheal tube and

the exhaled air was collected and analysed by gas chromatography for its isoprene content. The inhaled concentrations were in the range between 0.40 and 0.60 µg/ml (equivalent to 143200 and 214800 ppm), if not stated otherwise. The uptake of isoprene was not influenced by the ventilatory rate. Total retention by the lung thus ranged from 63.8 to 67.4% at ventilatory rates of between 6 and 30. In the animals in which only the lower respiratory tract was exposed by means of the endotracheal tube, the retained percentage of 71% was measured regardless of the ventilatory rate. Upper respiratory tract retention was found to be approx. 75%. In addition, the effect of isoprene concentration on retention was investigated. At concentration levels of 0.36 µg/ml, 0.51 µg/ml, 0.72 µg/ml and 0.96 µg/ml, the percentages of isoprene retention were 63.9%, 72%, 69% and 39.5%, respectively. Tidal volume showed no effect on the retention of isoprene in the lung, either. At tidal volumes of between 112 and 218 ml, the percentage of inhaled isoprene to be retained ranged between 74 and 76% (Egle and Gochberg, 1975).

7.2 Acute and subacute toxicity

Acute toxicity

• Oral administration

Groups of 15 male Wistar rats (weighing 160 to 210 g) received by oral gavage single doses of isoprene in the range from 250 to 2500 mg/kg body weight as a formulation in vegetable oil. The observation period was 14 days. The LD_{50} value was 2125 (between 2403 and 2210) mg/kg body weight. Observed clinical signs of toxicity included sedation and breathing difficulties, which developed within one hour of administration and continued for up to 7 days. Deaths occurred within 24 hours (Bayer, 1972).

• Dermal application

To ascertain the LD_{50} for a single dermal application, 1000 µl isoprene/kg body weight were applied to the dorsal skin of 5 male Wistar rats which had been shaved on the previous day. The chemical was not removed from the skin for 7 days. Signs of toxicity and deaths were not observed during the

14-day observation period which followed. Thus the LD_{50} value was > 1000 µl/kg body weight (equivalent to > 681 mg/kg body weight; Bayer, 1972).

• Inhalation administration

Groups of 20 male Wistar rats (weighing from 160 to 210 g) were exposed to isoprene concentrations ranging from 27600 to 100900 mg/m³ air for 4 hours, or to a concentration of 51500 mg/m³ for one hour. Groups of 20 female Wistar rats were exposed to concentrations between 29500 and 98100 mg/m³ air for 4 hours. The LC_{50} values found for male rats were > 100900 mg/m³ air and > 51500 mg/m³ for the 4-hour and 1-hour exposures, respectively, the value for the female rats being > 98100 mg/m³ air. Transient general malaise was the only sign of toxicity and it was seen only in the rats exposed to the highest concentrations (Bayer, 1972).

Following a 4-hour exposure to isoprene, the LC_{50} value in rats is reported to be 180000 mg/m³ (no further details; Shugaev, 1968, 1969).

Groups of 20 male NMRI mice (weighing 18 to 22 g) were exposed to isoprene vapours at concentrations of 14100 and 31500 mg/m³ air for 4 hours. None of the animals died. The LC_{50} value was > 31500 mg/m³ (Bayer, 1972).

Mice (10 animals/group) were exposed to isoprene at analytically ascertained concentrations ranging from 50000 to 150000 mg/m³ for 2 hours. The LC_{50} was given as 150000 mg/m³. The observation period was 21 days. The clinical signs of toxicity included irritation of the mucous membranes of the upper respiratory tract, impaired co-ordination, lying on the side and narcosis. At necropsy, severe hyperaemia of the internal organs and the brain was found (Korbakova and Fedorova, 1964).

Further LC_{50} values have been reported for 2-hour exposures of male mice and female mice to isoprene levels of 139000 mg/m³ and 148000 mg/m³, respectively (Gostinskii, 1965), and 2-hour exposure to 157000 mg/m³ (no information on the sex of the animals used; Shugaev, 1968, 1969). The chemical had irritating and narcotic effects. Macroscopic examination revealed that the lungs were enlarged and congested. Following several 2-hour exposures to isoprene, the concentration at which 50% of the exposed mice showed narcosis was 109000 mg/m³ (no further details; Gostinskii, 1965).

It has been reported that in mice concentrations in the 100000 to 120000 mg/m³ range caused deep narcosis and that 140000 mg/m³ were lethal (no further details; von Oettingen, 1940).

In 6 rabbits exposed to 4100 mg isoprene/m³ for a 40-minute exposure period a diminished, but quicker-acting flexor reflex was observed. The respiratory rate was increased by between 16.3 and 40% at a concentration of about 190 mg/m³ (Gostinskii, 1965).

In order to determine the total amount of inhaled ^{14}C-labelled isoprene in the toxicokinetic studies described in Section 7.1, the respiration of 5 male B6C3F1 mice (approx. 28 g, 12 to 13 weeks old) was investigated with the aid of a plethysmographic system. Breathing frequency, tidal volume and minute volume were measured at 1-minute intervals for the duration of the 6-hour exposure to 0 (controls), 20, 200 and 2000 ppm isoprene (equivalent to 0, 46, 460 and 4600 mg/m³). The results are shown in Table 10.

Table 10. Effect of isoprene exposure on respiration of mice

Isoprene concentration (ppm)	Respiratory rate (breaths/minute)	Tidal volume (ml/breath)	Minute volume (ml/minute)
0	347 ± 6	0.204 ± 0.023	70.7 ± 7.6
20	262 ± 9*	0.346 ± 0.028*	89.8 ± 5.4
200	307 ± 16	0.220 ± 0.017	68.2 ± 8.5
2000	244 ± 29*	0.227 ± 0.022	54.6 ± 6.2*

* Significantly different from the controls

Breathing frequency was depressed at all three concentrations, with statistically significant depression seen at 20 and 2000 ppm. At the high concentration the minute volume was significantly diminished, a finding which was not observed in rats exposed to levels of up to 8200 ppm (cf. Section 7.1; Dahl et al., 1987; Bond et al., 1991).

• Parenteral administration

Groups of 15 male Wistar rats (weighing 160 to 210 g) received single intraperitoneal doses of isoprene, as a formulation in vegetable oil, at dose levels ranging from 100 to 1750 mg/kg body weight. An LD_{50} value of 1390

(1310 to 1470) mg/kg body weight was ascertained. As in the oral administration studies, sedation and breathing difficulties were observed as the signs of toxicity (Bayer, 1972).

It has been reported that in rabbits subcutaneous injection of 1 ml isoprene had no effect on the erythrocyte count but did cause leucocytosis. According to the author, there were signs of irritation and “anaemia” of the bone marrow. The urine was found to contain traces of albumin as well as urobilinogen (no further details; von Oettingen, 1940).

Subacute toxicity

Groups of 30 male Wistar rats were investigated for the cumulative effect of isoprene in a modified Lim test (Lim et al., Arch. Int. Pharmacodyn., 130, 336 (1961)). In this study, a dose of 200 mg isoprene/kg body weight was administered orally to 30 rats on the first day of the study. The dose was increased by a factor of 1.5 on the following 4 days. The observation period was 7 days. None of the animals died. This study did not demonstrate any detectable cumulative effect of isoprene in male rats (no further details; Bayer, 1972).

Groups of 10 male and 10 female Wistar rats (weighing from 160 to 210 g) were exposed to isoprene vapour for 4 hours daily for 5 days at concentrations of 12700 or 57300 mg/m³. Both sexes tolerated the high concentration without any harmful effects (no further details; Bayer, 1972).

Six-hour exposures to isoprene vapour at a concentration of 6000 ppm (equivalent to 16740 mg/m³) were tolerated without clinical symptoms by 2 male and 2 female Alderley Park rats (weighing approx. 200 g). Autopsy revealed slight congestion of the lungs. Histopathological examination of the organs under investigation was without findings. Similarly, 15 6-hour exposures to 1670 ppm (equivalent to 4659 mg/m³) caused no clinical signs of toxicity in the 2 male and 2 female rats studied. Autopsy was without findings (no further details; Gage, 1970).

Groups of 20 male and 20 female F344 rats and 20 male and 20 female B6C3F1 mice inhaled isoprene vapour (purity: > 99%) at concentrations of 0 (controls), 438, 875, 1750, 3500 and 7000 ppm (equivalent to 0, 1222, 2441, 4883, 9765 and 19530 mg/m³) for 6 hours daily, 5 days per week for

2 weeks. After day 4 (rats) and day 5 (mice), haematology and clinical chemistry parameters were determined in 10 animals per species. All rats survived to the end of the study, and no effects were seen with respect to body weight gain, clinical signs, altered haematology or clinical chemistry parameters, or macroscopic or microscopic changes. Similarly, all mice survived to the end of the study. Body weight development was affected at the highest concentration in male mice. Treatment-related findings included slight increases in liver weights, decreases in thymus, spleen and testis weights as well as changes in haematology parameters (lowerings in erythrocyte count, haemoglobin concentration and erythrocyte mean corpuscular volume (MCV)). Microscopic examination revealed thymic and testicular atrophy (at 7000 ppm) and olfactory epithelial degeneration in the nasal cavities (at 1750 ppm and higher) of the males as well as epithelial hyperplasia of the forestomach (at all exposure levels) in both sexes (Melnick et al., 1990; NTP, 1995).

It has been reported that in mice 20 exposures of 2 hours each to an isoprene concentration of 60000 mg/m³ did not produce any narcotic effects. Upon necropsy the animals showed irritation of the bronchi and some emphysema of the lungs. Hyperplasia of the bone marrow was seen as well as signs of disintegration of the red blood cells in the spleen (pigmentation, macrophages; no further details; von Oettingen, 1940).

7.3 Skin and mucous membrane effects

Two New Zealand white rabbits (weighing from 2.8 to 3.8 kg) each had one ear brushed with isoprene twice a day on 5 consecutive days. Only a transient reddening of the skin occurred, which was regarded by the authors as a low grade skin-damaging effect (Bayer, 1972).

Application of 0.5 ml isoprene to the shorn skin of one rabbit caused extensive hyperaemia, oedema formation and subsequent desquamation (no further details; Korbakova and Fedorova, 1964).

The tail skin of mice was wetted slightly with isoprene. In the course of 2 hours marked hyperaemia of the skin occurred, and on the following days necrosis of the tail tips developed (no further details; Korbakova and Fedorova, 1964).

7.4 Sensitisation

No information available.

7.5 Subchronic and chronic toxicity

In a 13-week study, 10 male and 10 female F344 rats and 10 male and 10 female B6C3F1 mice (initially aged 6 to 8 weeks) inhaled isoprene (purity: > 99%) vapour at concentrations of 0 (controls), 70, 220, 700, 2200 and 7000 ppm (equivalent to 0, 195, 614, 1953, 6138 and 19530 mg/m³) for 6 hours (allowing an extra 12 minutes for attainment of 90% of the target concentration) daily, 5 days per week (whole-body exposure). Per group, 10 supplemental animals were exposed for haematology and clinical chemistry determinations on days 4 and 24, as were 5 animals each from the control, 70, 700 and 7000 ppm groups, in which the glutathione and total sulfhydryl concentrations in the liver, lung, kidney and thymus were determined 1 day and 12 weeks after exposure. During study week 12, the rats' urine was collected and analysed for glucose and creatinine levels as well as alkaline phosphatase and aspartate aminotransferase activities. Sperm motility and vaginal cytology were evaluated at the end of the study in the rats and mice exposed to 70, 700 or 7000 ppm (see also Section 7.8). Bone marrow samples were examined after 24 and 90 days. At the end of the study, gross pathology was performed on all animals, and the animals of the control and the 7000 ppm groups were examined microscopically. In the *rats,* no treatment-related changes were found with respect to survival, body weight gain, haematology, clinical chemistry and urinalysis parameters, organ weights as well as gross and microscopic examination. Glutathione levels in the examined organs of the exposed rats did not differ from those of the controls. Sperm motility and vaginal cytology evaluations were without findings. In the male *mice,* comparison with the controls at the end of the 13-week period did not reveal any changes with regard to body weight gain, while in the female mice, body weights were significantly lower than those of the control group at all levels of exposure. No clinical signs were observed during the study. Haematology at day 4 of the study revealed the occurrence in both sexes of a mild normocytic, normochromic anaemia (lowerings in the erythrocyte count as well as in haematocrit and haemoglobin concentration without affecting cell volume, reticulocyte count and polychromatic cells) at a concentration of 220 ppm. By

day 24 of the study, the anaemia became macrocytic (increased cell volume). The same finding was seen after 13 weeks. At the end of the study, the absolute and relative testis weights in the 2200 and 7000 ppm groups were significantly lower than those of the controls. The male mice of the highest concentration group were observed to have significantly higher absolute and relative liver weights, whilst in the female mice of the highest concentration group significantly higher absolute liver weights were seen. At the 700 ppm and higher, the absolute spleen weights were lower than those of the controls in both sexes, as were the relative spleen weights of the males at 220 ppm and higher, and of the females at 7000 ppm. The absolute kidney weights were found to be significantly increased in the female mice at 220 ppm and higher. The glutathione levels found in the lungs of the female mice on day 1 and in the livers and lungs of both sexes at the highest concentration in week 12 were lower than those in the controls. The total-sulfhydryl : glutathione ratio in these groups was higher than in the controls. Microscopic examination revealed changes in the forestomach (epithelial hyperplasia at 700 ppm and higher), the nasal cavity (olfactory epithelial degeneration in males 7000 ppm), the liver (hepatocellular hypertrophy at 2200 ppm and higher) and the testes (seminiferous tubule atrophy at 7000 ppm). Epididymal weights of mice exposed to isoprene levels of 700 to 7000 ppm were between 12 and 30% lower, sperm head count and sperm concentration were reduced by between 12 and 46% and sperm motility by from 6 to 23%, as compared with the controls. In the female mice of the 7000 ppm groups, the average oestrous cycle was significantly longer than in the females of the control group (Melnick et al., 1994; NTP, 1995).

Thirty-six mice, 11 rats and 5 rabbits inhaled isoprene for 4 hours/day during a period of 4 or 5 months (rats only). The isoprene concentrations in the inhalation chamber varied between 2200 and 4900 mg/m³ (nominal concentration: 5000 mg/m³). No information was reported with regard to the type and number of the control groups. There were no deaths. The mice were not observed to exhibit any effect on behaviour and the development of body weight during the study. A neuropharmacological assessment at the end of the study revealed an impaired performance in the swimming test (swimming times; exposed animals: 16,1 minutes, controls: 33,9 minutes). In the exposed rats the development of body weight corresponded to that seen in the controls. As of the third treatment month, a reduction in oxygen consumption was observed. At the end of the study, the controls

and the isoprene-treated group exhibited oxygen consumption values of 2210 ml/hour/kg body weight and 1860 ml/hour/kg body weight, respectively. The rabbits were found to have increased white blood cell counts as well as slightly decreased red blood cell counts; there was no effect on haemoglobin content. At necropsy, all animals were found to have increased lung, kidney and brain weights. Histopathological examination revealed degenerative changes in the liver in some animals. In rats and rabbits, bronchial irritation and damage to the lung (increased numbers of goblet cells in the bronchial mucosa, infiltration of bronchial wall by inflammatory cells, damage to the bronchial epithelial cells, vasculitis with perivascular oedema) were observed; in addition, “irritation” of the thyroid gland was seen in the rats, while the rabbits exhibited damage to the myocardium (interstitial infiltration, loss of cross-striation; no further details; Gostinskii, 1965). Insufficient documentation of the experimental setup and the results render the study suitable only to a limited extent for the assessment of systemic toxicity following repeated administration.

Fifteen rats were exposed to isoprene concentrations ranging from 200 to 600 mg/m³ for 5 hours daily on 6 days a week during 6 months. Fifteen rats served as controls. During the study, no behavioural changes or signs of neurotoxicity were observed. The development of body weight was normal. After 2 months, a decrease in urinary hippuric acid levels was observed which had returned to control values by the end of the study. Neither renal function nor haematological parameters were affected. Histological examination at the end of the study revealed purulent bronchitis, hyperplasia of the bronchial lymph nodes, thickened interalveolar septa, emphysema, fatty dystrophy of the liver cells, histiocytic infiltration of the kidney and the heart as well as haemosiderin deposits in the spleen (Korbakova and Fedorova, 1964). Insufficient documentation of the experimental setup and the results render the study suitable only to a limited extent for the assessment of systemic toxicity following repeated administration.

7.6 Genotoxicity

7.6.1 In vitro

Isoprene (purity: > 99%) was tested for its mutagenic potential in the Salmonella/microsome assay (preincubation assay) using *Salmonella typhi-*

murium strains TA 98, TA 100, TA 1535 and TA 1537 with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat and Syrian hamster liver). Concentrations of 100, 333, 1000, 3333 and 10000 µg/plate were employed. Dimethyl sulfoxide (DMSO) served as the solvent. At the highest concentration level, isoprene was toxic to the bacteria. The chemical proved to be devoid of mutagenicity in this test system, both in the presence and the absence of metabolic activation (NTP, 1983, 1995, 1997; Mortelmans et al., 1986).

Similarly, no indications of isoprene (purity: 99%) possessing any mutagenic properties were found in a further Salmonella/microsome assay, carried out as a desiccator test in the *Salmonella typhimurium* strains TA 98, TA 100, TA 1530, TA 1535 and TA 1538 with and without metabolic activation (S-9 mix from Aroclor-induced rat liver). Atmospheric concentrations of 25% v/v (75% v/v in the case of strain TA 1530) were tested. The exposure period was 24 hours (De Meester et al., 1981).

Isoprene also gave negative test results in the *Salmonella typhimurium* strains TA 102 and TA 104 (no further details; Kushi et al., 1985).

The following isoprene metabolites were tested without metabolic activation for their mutagenic potential in the *Salmonella typhimurium* strains TA 98 and TA 100:

- I 3,4-epoxy-3-methyl-1-butene (purity: 95%)
- II 3,4-epoxy-2-methyl-1-butene (no information on purity)
- III 2-methyl-1,2,3,4-diepoxybutane (purity: 99%)

The metabolites were used at concentrations ranging from 2 to 30 mM/plate (equivalent to 136 to 2043 µg/plate). At the high concentration, a bacteriotoxic effect was observed. Metabolites I and II did not show any mutagenic properties. At concentrations of about 5 mM/plate (equivalent to 341 µg/plate) and higher, metabolite III caused concentration-dependent increases in revertant counts in both of the strains. At the 5 mM/plate level, the revertant counts in the strains TA 98 and TA 100 showed 1.5-fold and 2.4-fold increases, respectively, over the controls; at the 10 mM/plate (equivalent to 681 µg/plate) level, increases were 2.8-fold and 9.4-fold, and at the 15 mM/plate (equivalent to 1022 µg/plate) level 1.5-fold and 11.5-fold over the control values, respectively. Marked alkylating activity was found for metabolite III in an in vitro test procedure in which nicotinamide served

as the nucleophilic agent. The alkylation rates measured for metabolites I, II and III were 15, 35 and 360, respectively (Gervasi et al., 1985; Gervasi and Longo, 1990).

In the SCE test, isoprene (purity: > 99%; solvent DMSO) concentrations ranging from 50 to 1600 µg/ml in the absence of metabolic activation (26-hour exposure) and from 160 to 5000 µg/ml in the presence of metabolic activation (S-9 mix from Aroclor 1254-induced livers from male Sprague-Dawley rats; 2-hour exposure) did not induce any increase in the frequency of sister chromatid exchange in CHO cells. Positive controls were treated with mitomycin C and cyclophosphamide, respectively. In both cases, the number of evaluated cells was 50. No information was given with regard to cytotoxicity (NTP, 1995, 1997).

In the chromosome aberration test in CHO cells, isoprene (purity: > 99%; solvent DMSO) gave negative test results both with and without metabolic activation (S-9 mix from Aroclor 1254-induced livers from male Sprague-Dawley rats) at concentration levels ranging from 1600 to 5000 µg/ml. The exposure periods without metabolic activation were 10 hours, whereas those with metabolic activation were 2 hours. Positive controls were treated with mitomycin C and cyclophosphamide, respectively. The number of evaluated cells was 200. No information was given with regard to cytotoxicity (NTP, 1995, 1997).

7.6.2 In vivo

In the carcinogenicity study in male and female F344 rats which is discussed in Section 7.7 (NTP, 1997), additional groups of 10 animals/sex were exposed to isoprene (purity: 99%) at concentrations of 0 (controls), 220, 700 or 7000 ppm (equivalent to 0, 614, 1953 or 19530 mg/m³) for 6 hours daily, 5 days per week for 4 weeks. The rats each received a total of 17 to 19 exposures with at least two consecutive exposures before sacrifice and subsequent isolation of the lung fibroblasts. Per rat, 1000 lung fibroblasts were examined for micronuclei. No elevated frequencies of micronucleated lung fibroblasts were found. Positive controls were carried out (NTP, 1997).

Groups of 15 male B6C3F1 mice (5 to 6 weeks old) were exposed to isoprene (purity: > 98%) at concentrations of 438, 1750 and 7000 ppm

(equivalent to 1222, 4883 and 19530 mg/m³) for 6 hours per day on 12 exposure days (3 exposure days, 2 non-exposure days, 5 exposure days, 2 non-exposure days, 4 exposure days). Fifteen controls inhaled air. Approximately one hour prior to the 12th exposure, a 50 mg bromodeoxyuridine tablet was implanted subcutaneously. For analysis of chromosome aberrations, 10 of the exposed mice were killed between 17 and 20 hours after tablet implantation, whilst the other 5 mice were killed 24 hours after implantation for analysis of the frequency of sister chromatid exchange. Two hours prior to kill time, each animal was injected intraperitoneally with 2 ml colchicine per kilogram body weight. At the same time, peripheral blood smears were prepared from each animal (tail vein) and analysed for micronucleated erythrocytes. On sacrifice, the bone marrow from each animal was prepared and examined for chromosome aberrations and frequency of sister chromatid exchange. At the administered levels, isoprene exposure induced a significant increase in the frequency of sister chromatid exchange in bone marrow cells and of micronucleated erythrocytes in peripheral blood. In addition, exposure resulted in a lengthening of the average generation time of bone marrow cells at the highest concentration as well as a dose-dependent reduction in polychromatic erythrocytes in the peripheral blood. However, no indications were found of a potential to cause chromosome aberrations. Similarly, exposure to isoprene did not significantly alter the mitotic index in bone marrow (Tice et al., 1988; Shelby, 1990; NTP, 1995, 1997).

In the context of a carcinogenicity study with inhalation administration of isoprene to B6C3F1 mice (cf. Section 7.7; Placke et al., 1996; IISRP, 1993) for exposure periods of 8 hours per day for 40 weeks (at 70, 140 and 2200 ppm) and 80 weeks (at 10, 70, 280, 700, 2200 (4-hour exposures) and 2200 ppm), in each case the peripheral blood of 10 animals was analysed for micronuclei according to the method published by Miller (1973). Per level, 10 animals served as controls. Evaluations were carried out for 2000 cells per animal. After 40 weeks, no differences were seen between the controls and the animals which had been exposed to 70 and 140 ppm. The frequency of micronuclei in the mice of the 2200 ppm groups was significantly elevated (by 147%) as compared with the controls. Following an exposure period of 80 weeks, the animals of the 10 and 70 ppm groups exhibited no differences compared with the controls. All mice which had been exposed to concentrations $\geq$ 280 ppm were found to have significantly

higher micronucleus scores than the controls (by 92%, 133%, 167% and 125% at 280 ppm, 700 ppm, 2200 ppm (4 hours) and 2200 ppm, respectively; IISRP, 1993; Placke et al., 1996). The increase in the frequency of micronucleated erythrocytes in peripheral blood as described above (Tice et al., 1988; Shelby, 1990; NTP, 1995) thus was confirmed by the study.

The Harderian gland neoplasms found in male B6C3F1 mice after a 6-month exposure to isoprene concentrations of 2200 and 7000 ppm (equivalent to 6138 and 19530 mg/m³) and a subsequent observation period of 6 months (for a detailed description of the study, cf. Section 7.7; Melnick et al., 1992, 1994, 1996; NTP, 1995) were characterised by genetic alterations in the K-*ras* and H-*ras* proto-oncogenes. The K-*ras* and H-*ras* mutations were identified by single strand conformation polymorphism analysis and direct sequencing of DNA which had been amplified by the polymerase chain reaction upon its isolation from the neoplasms. A high frequency of K-*ras* and H-*ras* mutations was detected in the isoprene-induced neoplasms (100%) as compared with neoplasms from controls (0%) or with historical control data for B6C3F1 mice (65%). The predominant mutations consisted in A to T transversions (CAA to CTA) at the K-*ras* codon 61 (15/30) and C to A transversions (CAA to AAA) at the H-*ras* codon 61 (8/30). In the authors' opinion, these data suggest that activation of *ras* proto-oncogenes contributes to Harderian gland tumorigenesis in B6C3F1 mice exposed to isoprene (no further details; Hong et al., 1995).

7.7 Carcinogenicity

Groups of 40 male F344 rats were underwent whole-body exposure to isoprene (purity: > 99%) vapour concentrations of 0 (controls), 70, 220, 700, 2200 or 7000 ppm (equivalent to 0, 195, 614, 1953, 6138 or 19530 mg/m³) for 6 hours daily (allowing an extra 12 minutes for attainment of 90% of the target concentration), 5 days per week for 26 weeks. Following the 26-week exposure, 10 animals/group were investigated with regard to haematological parameters as well as receiving gross and microscopic examination; the remaining 30 animals/group were placed under observation for 26 weeks post exposure. In the exposed rats, body weight development did not differ from that of the controls. One rat of the 220 ppm group died during the exposure period (no indication of the cause of death). No clinical signs of toxicity were observed during the study. At the end of the exposure

period, no changes were seen in the measured haematology parameters when comparing exposed rats with controls. The absolute liver weights were significantly higher than controls at the highest concentration, while the absolute kidney weights were increased at all exposure levels. The organ weight increases were reversible at the end of the observation period. In the testis, an increased incidence of interstitial cell hyperplasia of varying degree was detected at 700 ppm and higher at the end of the 26-week exposure (control 10%, 7000 ppm 100%). According to the authors, such instances of hyperplasia are unusual in rats 6 months of age, for which reason they were considered treatment-related. At the end of the observation period, an elevated incidence of interstitial cell adenomas was observed (Melnick et al., 1992, 1994, 1996; NTP, 1995).

In a further carcinogenicity study in F344 rats, groups of 50 males and 50 females were exposed to isoprene (purity: 99%) vapour at concentrations of 0 (controls), 220, 700 or 7000 ppm (equivalent to 0, 614, 1953 or 19530 mg/m³) for 6 hours daily (allowing an extra 12 minutes for attainment of 90% of the target concentration), 5 days per week for 104 weeks. After 3, 6, 12 and 18 months, the urine of 10 males and 10 females per group were analysed for urine weight and the concentrations of creatinine and 2-hydroxy-2-methyl-3-butenenoic acid. According to the authors, 2-hydroxy-2-methyl-3-butenenoic acid, which is an oxidation product of the isoprene metabolite, *trans*-3-methyl-2-butene-3,4-diol, is a biomarker of isoprene exposure (see also Section 7.1; NTP, 1994). In all groups exposed to isoprene, the concentration of 2-hydroxy-2-methyl-3-butenenoic acid increased with increasing isoprene concentrations. However, the increases in 2-hydroxy-2-methyl-3-butenenoic acid levels normalised to urine creatinine were not proportional to isoprene exposure concentrations, indicating in the authors' opinion that metabolism was nonlinear in the concentration range investigated. Survival rates and body weight development of the rats exposed to isoprene were similar to those seen in the controls. The incidences of tumours are shown in Table 11. The male mice were found to have increased incidences of mammary gland fibroadenoma at the highest concentration, while in the female mice incidences of mammary gland fibroadenoma were increased even at the lowest test concentration of 220 ppm. Increases were also seen in the males at the 700 ppm level and above with respect to interstitial cell adenoma of the testis and renal tubule adenoma as well as renal tubule hyperplasia. In the brain of male mice and

female mice, 2 and 7 neoplasms were found, respectively. These included malignant and benign astrocytoma, malignant glioma and medulloblastoma, meningeal granular cell tumour and meningeal sarcoma, neoplasms which were very rare or had never occurred in historical controls and therefore, in the authors' opinion, were also possibly treatment-related. Non-neoplastic changes observed in the male rats of the 700 and 7000 ppm groups included an increased incidence of spleenic fibrosis. The male rats of the high concentration group showed an increased incidence of parathyroid gland hyperplasia, which according to the authors was commonly observed in male rats with nephropathy. Based on the increased incidence in male rats of tumours of the mammary glands, renal tubules and the testis, there was clear evidence of carcinogenic activity of isoprene in male rats. In female rats, there was some evidence of carcinogenic activity on account of the increased incidence of mammary gland fibroadenoma. The occurrence of very rare brain tumours in the female rats was also interpreted by the authors as a possible consequence of treatment (NTP, 1997).

Table 11. Incidence of tumours in male and female F344 rats after a 104-week exposure to isoprene

	Concentration (ppm)							
	0		220		700		7000	
	♂	♀	♂	♀	♂	♀	♂	♀
Mammary gland								
Fibroadenomas	2/50 (4%)	19/50 (38%)	4/50 (8%)	35/50* (70%)	6/50 (12%)	32/50* (64%)	21/50* (42%)	32/50* (64%)
Carcinomas	0/50	4/50	1/50	2/50	1/50	1/50	2/50	3/50
Fibroadenomas and Carcinomas	2/50 (4%)	20/50 (40%)	5/50 (10%)	35/50* (70%)	7/50 (14%)	32/50* (64%)	21/50* (42%)	32/50* (64%)
Kidney								
Renal tubule adenomas or Carcinomas	2/50 (4%)	0/50	4/50 (8%)	0/50	8/50* (16%)	5/50	15/50* (30%)	0/50
Testis								
Adenomas	33/50 (66%)	–	37/50 (74%)	–	44/50* (88%)	–	48/50* (96%)	–
Brain								
Malignant or benign astrocytomas	0/50	0/50	0/50	0/50	0/50	1/50	1/50	0/50
Malignant gliomas								
Malignant medulloblastomas	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
Meningeal benign	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
granular cell tumours	0/50	0/50	0/50	1/50	1/50	0/50	0/50	1/50
Meningeal sarcomas								
	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50

* Significantly different from the control group

As described in the 26-week rat study (cf. Melnick et al., 1992, 1994, 1996; NTP, 1995) summarised above, groups of 40 male B6C3F1 mice underwent whole-body exposure to vaporous isoprene (purity: > 99%) for 6 hours per day (allowing an extra 12 minutes for attainment of 90% of the target concentration), 5 days a week for 26 weeks at vapour concentrations of 0 (controls) 70, 220, 700, 2200 and 7000 ppm (equivalent to 0, 195, 614, 1953, 6138 and 19530 mg/m³). After the 26-week exposure, 10 animals per group were examined macroscopically and histopathologically as well as with respect to haematological parameters, the remaining 30 animals/group being kept under observation for a post-exposure period of 26 weeks. During the exposure period, one mouse per group died in the control group and the 70 ppm and 700 ppm groups, while 2 mice of the 2200 ppm group and 6 mice of the 7000 ppm group or were killed *in extremis*. During the post-exposure observation period, the number of deceased mice per group was 1 in the 70 ppm group, 2 in the 0, 220, 700 and 2200 ppm groups, and 3 in the 7000 ppm group. Body weights in the highest concentration group were significantly lower than those of the control group after the 26-week exposure, but at the end of the observation period they were back in the range of the controls. Clinical signs of toxicity seen towards the end of the exposure period at the highest exposure level included abnormal posture and hindlimb paralysis, both of which were reversible in the course of the observation period (results of more detailed neurotoxicity studies are extensively discussed in Section 7.10). At exposure levels of 700 ppm and above, macrocytic anaemia occurred at the end of the exposure period, as it had done in the 13-week inhalation study (cf. Section 7.5). At the highest level, the absolute and relative liver weights found at the end of the exposure and post-exposure observation periods were significantly increased as compared with the controls. At the end of the observation period the relative liver weights of the 700 ppm and 2200 ppm groups were also increased. A decrease in absolute and relative testicular weights was seen in the top concentration group at the end of the exposure period, a finding which was reversible at the end of the study. At both time points of examination, the animals exposed to 7000 ppm showed lower absolute brain weights as compared with the controls; at the end of the observation period, the relative brain weights at 700 ppm and higher were significantly lower than those of the controls. At the end of the study, mice exposed to levels of 700 ppm and above had a higher incidence of tumours of the liver, lung, forestomach and Harderian gland (cf. Table 12). Moreover, at this

concentration level and above, the incidence of multiple and/or malignant neoplasms was higher than in the controls.

Table 12. Incidence of tumours in male B6C3F1 mice after a 26-week exposure to isoprene followed by a 26-week observation period						
	Concentration (ppm)					
	0	70	220	700	2200	7000
Liver (hepatocellular adenomas or carcinomas)	7/30 (23%)❶	3/30 (10%)	7/29 (24%)	15/30 (50%)❷	18/30 (60%)❷	17/28 (61%)❷
Lung (alveolar/bronchiolar adenomas or carcinomas)	2/30 (7%)	2/30 (7%)	1/29 (3%)	5/30 (17%)	10/30 (33%)❷	9/28 (32%)❷
Forestomach (squamous cell papillomas or carcinomas)	0/30 (0%)	0/30 (0%)	0/30 (0%)	1/30 (3%)	4/30 (13%)	6/30 (20%)❷
Harderian gland (adenomas)	2/30 (7%)	6/30 (20%)	4/30 (13%)	14/30 (47%)❷	13/30 (43%)❷	12/30 (40%)❷

❶ Number of animals with neoplasms/Number of animals examined
❷ Significantly different from the control group

Non-neoplastic changes seen included changes in the testes and atrophy of the seminiferous tubules in the 7000 ppm groups, epithelial hyperplasia of the forestomach at 700 ppm and higher as well as mild olfactory epithelial degeneration and macrocytic anaemia at 220 ppm and higher. In mice, the results provide clear evidence of carcinogenicity for isoprene (Melnick et al., 1992, 1994, 1996; NTP, 1995).

A further carcinogenicity study with inhalation exposure was carried out in male and female B6C3F1 mice (50 animals/group). Isoprene concentrations (purity: ≥ 99%) ranged from 10 to 2200 ppm (equivalent to 28 to 6138 mg/m³). The exposure periods were 4 or 8 hours daily, 5 days per week for 20, 40 or 80 weeks (whole-body exposure). Subsequently, the animals were placed under observation up to study week 104. The exposure regimen for this study is shown in Table 13.

Table 13. Exposure regimen for a chronic isoprene inhalation study in B6C3F1 mice lasting up to 80 weeks				
Concentration		Number of animals	Daily exposure period (hours)	Duration of study (weeks)
(ppm)	(mg/m³)			
0	0	50 ♂, 50 ♀	8	80
10	28	50 ♂, 50 ♀	8	80
70	195	50 ♂, 50 ♀	8	80
70	195	50 ♂	8	40
140	391	50 ♂	8	40
280	781	50 ♂	8	80
280	781	50 ♂	8	20
700	1953	50 ♂	8	80
2200	6138	50 ♂	8	80
2200	6138	50 ♂	8	40
2200	6138	50 ♂	4	80
2200	6138	50 ♂	4	20

During the first 13 weeks, the body weights were recorded on a weekly and thereafter on a monthly basis. At the end of the study, haematology parameters (red blood cell count, haematocrit, haemoglobin, white blood cell count, differential blood count and morphology) were determined and a complete gross and histopathological examination performed on all animals. There was an isoprene concentration-related effect on survival. At approx. week 80, the animals which had been exposed to concentrations ≥ 280 ppm had lower survival rates (< 50%; no further details). These animals were therefore sacrificed in study week 96 so as to ensure an adequate number of animals for histopathology. Body weight development in all exposed groups corresponded to the development seen in the controls. Clinical observations included treatment-related swelling of the abdomen as early as study day 56 in the 2200 ppm groups. The frequency of the abdominal swelling correlated directly with the incidence of liver tumours. Swelling of the eye was first observed on study day 534 and correlated with the incidence of Harderian gland tumours. No isoprene-related effects on the evaluated haematology parameters were observed. In those animals which were exposed to isoprene concentrations of 280, 700 and 2200 ppm for 80 weeks, absolute and relative testis weights were significantly less (by 20 to 30%) as compared with the controls. In the females, ovary

weights in the two exposure groups (10 and 70 ppm) were not significantly less, compared with the controls. The incidences of tumours in the male mice is summarised in Table 14.

Table 14. Incidences of tumours in male B6C3F1 mice after 20, 40 and 80 weeks of isoprene exposure (total study duration 96 or 104 weeks)

ppm/Weeks	0/80	10/80	70/40	70/80	140/40	280/20	280/80	700/80	2200/20❶	2200/80❶	2200/40	2200/80
ppm × Weeks	0	800	2800	5600	5600	5600	22400	56000	22000	88000	88000	176000
Alveolar/bronchiolar												
adenomas	11/50	16/50	8/50	4/50	10/50	16/50	13/50	23/50❷	14/50	15/50	29/49❷	30/50❷
carcinomas	0	1	0	2	1	3	1	7❷	2	3	3	7❷
Hepatocellular												
adenomas	11/50	12/50	14/49	5/50	22/50❷	18/49	24/50❷	27/48❷	22/50❷	21/50❷	28/47❷	30/50❷
carcinomas	9	6	11	9	10	12	16	17	12	15	18❷	16
Harderian gland												
adenomas	4/47	4/49	13/48❷	9/50	12/50❷	16/49❷	17/50❷	26/49❷	19/49❷	28/50❷	31/49❷	35/50❷
carcinomas	0	0	0	0	2	3	1	3	1	2	0	2
Haemangio-sarcomas												
heart	0/49	0/50	0/49	0/50	0/50	0/50	2/50	1/50	4/50	1/50	1/49	1/50
spleen	1/49	3/48	1/47	2/50	3/50	2/47	1/50	2/48	2/48	2/50	0/47	1/49
Forestomach squamous												
papillomas	0/50	0/48	0/47	0/50	0/49	0/46	0/50	1/47	0/48	1/50	2/47	1/50
carcinomas	0	0	0	0	0	0	1	0	1	1	0	3
Histiocytic sarcomas	0/50	2/50	2/50	2/50	1/50	8/50❷	4/50	2/50	5/50❷	7/50❷	7/50❷	2/50
Lymphomas	2/50	1/50	2/50	4/50	1/50	7/50	5/50	4/50	4/50	4/50	5/50	6/50

❶ Exposure for 4 instead of 8 hours/day
❷ Significantly different from the controls ($p < 0.05$; Fisher's Exact Test)

At exposure concentrations of 140 ppm and higher, and of 70 ppm and higher, the incidences of hepatocellular adenomas and of Harderian gland adenomas, respectively, were significantly increased. The incidence of primary alveolar adenomas and carcinomas was significantly increased in the groups exposed to 700 or 2200 ppm isoprene for 8 hours/day. Six mice exposed to concentrations in excess of 140 ppm were diagnosed with squamous cell carcinomas of the forestomach. A total of 5 squamous papillomas of the forestomach were found in animals of the two highest concentration groups. The incidence of histiocytic sarcomas observed at an exposure level of 280 ppm was increased in a concentration-independent manner. In the exposed male mice, a slightly increased incidence of haemangiosarcoma of the spleen and the heart were observed. According to the authors, cardiac haemangiosarcomas are extremely rare in B6C3F1 mice (0/658 in historical controls from chronic inhalation studies). In the females exposed to 10 and 70 ppm isoprene, the 70 ppm groups exhibited higher incidences of haemangiosarcomas of the spleen (4/50; controls

1/50), Harderian gland adenomas (8/49; controls 2/49) and pituitary adenomas (9/49; controls 1/49). Comparing their data with historical control values for haemangiosarcomas of the spleen (4/654; 0.61%), the authors considered the increased incidence of haemangiosarcomas seen in the 70 ppm groups (8%) to be possibly treatment-related. The increased incidences of Harderian gland and pituitary adenomas were in the range of the historical controls. The non-neoplastic changes seen in the male mice of the higher concentration groups included a slight increase in alveolar epithelial lining cell hyperplasia and focal areas of epithelial hyperplasia of the forestomach mucosa (no further details). The female mice of the 70 ppm group and the males of the 140, 280, 700 and 2200 ppm groups were found to have focal areas of mild metaplasia of the olfactory epithelium to respiratory epithelium. The exposed mice were observed to have increased incidences of degenerative changes in myocardial muscle, testis and epididymis as well as preputial gland (no further details), which the authors attributed to the poor general condition of the animals. Hindlimb paralysis and degenerative changes in the spinal cord, as observed in the National Toxicology Program (NTP) carcinogenicity study described above (see also Section 7.10), were not found. The *no observed effect level* observed in this study was 10 ppm (equivalent to 28 mg/m^3), and the *lowest observed effect level* for tumours was reported as 70 ppm (equivalent to 195 mg/m^3). The study design also made it possible to establish that cumulative exposure does not permit adequate prediction of tumour incidence. The same cumulative exposure appeared to be more or less toxic, depending on how it was administered over time. For instance, a 4-fold increase in isoprene concentration (from 70 to 280 ppm) with a concurrent reduction in exposure duration by an equal factor of 4 (from 80 to 20 weeks) resulted in a significant increase in lung tumours from 12% to 38% and in a 25% increase in liver tumours (IISRP, 1993; Placke et al., 1996; Cox et al., 1996).

Based on the results of the NTP carcinogenicity study in rats and mice (Melnick et al., 1992, 1994, 1996; NTP, 1995), the International Agency for Research on Cancer (IARC) classified isoprene as possibly carcinogenic to humans in 1994 (Group 2B; IARC, 1994). In further, more recently conducted carcinogenicity studies (IISRP, 1993; Placke et al., 1996; Cox et al., 1996; NTP, 1997), isoprene has also proved to be carcinogenic.

In a tumour initiation-promotion study, groups of 30 female ICR Swiss mice (55 to 60 days old) received single applications to the shaved dorsal skin of

0.125 mg 7,12-dimethylbenz[α]anthracene (DMBA) dissolved in 0.25 ml acetone. Following a treatment-free period of 3 weeks, the animals had 0.15% isoprene in 0.006% croton resin in acetone applied to the skin 5 times per week for 18 weeks. Appropriate treatment groups (DMBA + croton resin, DMBA, acetone) served as controls. The mice were examined weekly and the number and distribution of tumours noted. Body weights were recorded once a month. By the end of the study, 90% of the animals treated with isoprene and croton resin as well as the positive controls had developed tumours. Per mouse, 8.0 papillomas were identified (DMBA + croton resin: 12.2, DMBA: 0, acetone: 0; no further details; Shamberger, 1971).

7.8 Reproductive toxicity

Groups of 24 to 26 pregnant Sprague-Dawley rats underwent whole-body exposure to 0 (controls), 280, 1400 and 7000 ppm isoprene vapours (equivalent to 0, 781, 3906 and 19530 mg/m³; purity: > 99%) for 6 hours per day (allowing an extra 12 minutes for attainment of 90% of the concentration) on days 6 to 19 of gestation. Groups of 10 virgin females per exposure level received the same treatment. The body weights of the pregnant rats were determined on days 0, 6, 10, 14, 17 and 20, those of the non-pregnant rats on days 1, 5, 10 and 20. One day after the last exposure, the animals were killed, the liver, kidney and gravid uterus weighed, the number of implantations, resorptions and of live and dead foetuses per litter determined, and the placenta examined. The live foetuses were weighed and examined for visceral and skeletal malformations. None of the pregnant rats died during the study. No clinical signs of toxicity were observed. The body weight development of the pregnant and virgin rats corresponded to that of the controls at all time points investigated in the study. The organ weights of the pregnant rats did not differ from those of the controls, with the exception of relative kidney weight at the highest concentration (7000 ppm), which exhibited a slight, but statistically significant increase as compared with the controls ($p < 0.05$; Turkey's t-test). No statistically significant differences were noted between the exposed animals and the controls with regard to implantations per dam, resorptions per litter and foetal mortality and body weights. No increased incidence of malformations or higher percentage of malformations per litter was observed in the exposed animals.

The incidence and percentage of malformations per litter were not observed to be increased in the exposed animals. The incidence of foetuses with variations or reduced ossification was not increased in the exposed animals (Mast et al., 1989; NTP, 1995).

Employing the study design described above, groups of 27 to 29 pregnant CD1-Swiss mice were also exposed to isoprene on days 6 to 17 of gestation. The pregnant mice had their body weights determined on days 0, 6, 9, 12, 15 and 18, the virgin mice being weighed on days 1, 5, 10 and 18. None of the animals died during the study. No clinical signs of toxicity were seen. In the virgin mice, body weight development corresponded to that of the controls. In contrast, body weights of the exposed mice were lower compared with the controls on days 12, 15 and 18, the decrease being statistically significant at the highest exposure level (7000 ppm) on days 15 and 18. At the highest concentration, gravid uterine weight was significantly less than that of the control group. In the exposed mice, the absolute liver and kidney weights did not differ significantly from those of the controls. However, in the 1400 ppm groups the relative liver weights were higher than in the control group, and in the 7000 ppm groups the relative liver and kidney weights were significantly increased as compared with the controls. In the exposed animals the numbers of implantations per dam and of resorptions per litter did not differ from the controls. There were no statistically significant differences with respect to foetal mortality and number of live foetuses per litter. However, the body weights of male and female foetuses decreased with increasing exposure concentration. Body weights were significantly lower than controls in the male foetuses at the 1400 and 7000 ppm exposure levels and in the female foetuses at all three concentrations. There was no increase in overall incidence of malformations or percentage of malformed foetuses per litter. The incidence of variations and reduced ossifications rose from 22.3% in the controls to 40.3% in the 7000 ppm group. The percentage per litter of foetuses exhibiting variations/reduced ossification (predominantly supernumerary ribs) rose with increasing exposure concentration and at the highest concentration was significantly higher than that seen in the controls (control 24%, 7000 ppm 41.3%; Mast et al., 1989; NTP, 1995).

Wistar rats received oral doses of 22, 379 and 1895 mg isoprene/kg body weight from the 9th to the 12th day of pregnancy. There were no indications of embryotoxicity or teratogenicity. The foetuses exhibited slightly retarded

ossification in sternebrae and, to an even lesser extent, in occipital bone (no further details; Tsutsumi et al., 1969).

In the 13-week inhalation studies conducted within the National Toxicology Program (NTP) and described in Section 7.5, investigations of sperm motility and vaginal cytology in Fischer-344 rats exposed to 70, 700 or 7000 ppm isoprene did not reveal any findings. In contrast, the mice had lower absolute and relative testicular weights than the controls at the two highest concentrations (2200 and 7000 ppm). Histology revealed seminiferous tubule atrophy at 7000 ppm. In the males that were intended for investigation of sperm motility (70, 700 and 7000 ppm), the epididymal weights in the 700 and 7000 ppm groups were decreased by 12 and 30%, respectively, as compared with the controls. Sperm head count and sperm concentration in the 700 and 7000 ppm groups were 12 and 14% lower than the controls, respectively, while sperm motility was reduced by 6 and 23%, respectively. In the females exposed to the highest concentration average oestrus cycle length was significantly longer than in the control group (Melnick et al., 1994; NTP, 1995).

The rats exposed to isoprene in the NTP carcinogenicity studies described in Section 7.7 histologically exhibited an increased incidence of varying degrees of interstitial cell hyperplasia of the testis at the end of the 26-week exposure period. According to the authors, such hyperplasia is unusual in rats at 6 months of age and was therefore evaluated as being related to treatment. In the rats with a recovery period of 26 weeks after exposure, an increased incidence of interstitial cell adenomas was observed. The mice exposed to the highest concentration exhibited lower absolute and relative testicular weights at the end of the exposure period. Histological examination revealed seminiferous tubule atrophy in the animals exposed to the highest concentration. At the end of the observation period, testicular weights and incidence of seminiferous tubule atrophy had returned to values within the control range (Melnick et al., 1994; NTP, 1995).

In male rats exposed to isoprene (4000 mg/m³ for 4 hours), no changes were observed with respect to sperm count, percentage of live and motile spermatozoa, osmotic resistance and number of pathological spermatozoa 24 hours after cessation of exposure (no further details; Repina, 1988).

In order to investigate the potential ovarian toxicity of isoprene, 10 28-day-old female B6C3F1 mice were treated intraperitoneally with 7.34 mmol iso-

prene (no indication of purity)/kg body weight/day (equivalent to 500 mg/kg body weight/day) for 30 days. A control group of 10 mice received sesame oil (2.5 ml/kg body weight). On completion of treatment on day 30, the animals were killed on the first day of the dioestrus of their cycle. Their oestrual status was determined by means of vaginal cytology. The ovaries were examined histologically, with the small and growing preantral follicles being counted in every 20th section. Isoprene reduced the number of small follicles by 76 ± 5% (control group 131 ± 13, test group 31 ± 6) and the number of growing follicles by 46 ± 8% (control group 51 ± 4, test group 28 ± 4) and therefore proved to be toxic to the ovaries (Doerr et al., 1995).

7.9 Effects on the immune system

In the rat, a reduction in the number of cells and a lower mitotic index were found in the thymus 24 hours after a 4-hour exposure to isoprene. The exposure concentrations were 8400 ± 1090 and 21410 ± 1630 mg/m³. In addition, the absolute and relative thymus weights were significantly reduced. Three days after exposure, the values had returned to normal. Following exposure to lower isoprene concentrations (810 and 2180 mg/m³), an increase was seen in the mitotic index in the thymus as well as the lymphocyte count in the peripheral blood 24 hours after exposure (no further details; Mamedov, 1979).

Male Wistar rats were exposed to isoprene at concentrations of 98 ± 4 and 1016 ± 28 mg/m³ for 4 hours daily over a period of 30 days. Investigations on days 2, 4, 8, 15 and 30 of the study included determinations of the spleen and thymus weights, the number of cells and the mitotic index in the thymus, and the number of lymphocytes in the peripheral blood. No further information was given on the number of animals per group or the type of control group. During exposure, both dose groups showed phases in which a decrease in the number of cells as well as a significant inhibition of mitosis occurred in the thymus and significantly increased lymphocyte counts in the peripheral blood as well as a decrease in thymus weight and significant increase in spleen weight were found. After 30 days of exposure, the mitotic index in the thymus was increased in the low-dose group, while in the high-dose group both the number of cells and the mitotic index in the thymus was significantly reduced (no further details; Mamedov, 1979).

In addition, rats were exposed to isoprene concentrations of 116 ± 7 and 10 ± 0.15 mg/m³ for 4 hours daily over 4 months. Subsequently, the animals were placed under observation for one month. After 1, 2, 3, 4 and 5 months, the spleen and thymus weights, number of cells and mitotic index in the thymus were determined as well as the number of lymphocytes in the peripheral blood. Further investigations included the immunological reaction to sheep erythrocytes, the cell compositions of the inguinal lymph node and the bone marrow as well as the number of lymphocytes in the femur, and additionally, other “integral indices”, such as the “mink” reflex, orienting reflexes, et cetera. In the highest dose group, the number of cells in the thymus and thymus weight were found to be significantly reduced after 3 months, whereas they were increased after 4 months. There were also changes in the number of lymphocytes in the peripheral blood. At the end of the observation period, comparison of the exposed animals and a control group (no further details) did not reveal any differences. In the low-dose group, all of the investigated parameters were within the ranges of the controls. However, one month after the end of the study the proliferative activity of the thymus was found to be increased (no further details; Mamedov, 1979).

Rabbits (strain, age and weight not indicated) were exposed to an average concentration of 400 mg isoprene/m³ at room temperature or at 30 to 32 °C for 4 hours daily over a period of 4 months. A control group was used. Two weeks after commencement of the study and every 4 weeks thereafter, determinations were carried out to assess the bactericidal activity of the blood serum, complement titre, lysozyme activity, β-lysin activity, total nucleic acid content in blood, total protein, protein fractions in blood serum and phagocytic activity. In the animals exposed to isoprene at room temperature, a decrease in the activity of the immunological parameters was seen only towards the end of the study (no further details). In the rabbits exposed to isoprene at higher temperatures, activation of the protective properties (no further details) was observed at the beginning of the exposure phase, followed by normalisation and, at the end of the study, a decrease in lysozyme and phagocytic activity, nucleic acids and total proteins (Samedov et al., 1978; Faustov, 1972).

Insufficient documentation of the experimental setup and the results render the study unsuitable for assessment of the effects of isoprene on the immune system.

7.10 Neurotoxicity

The neurotoxic potential of isoprene was investigated in the NTP carcinogenicity study described in Section 7.7 (Melnick et al., 1992, 1994, 1996; NTP, 1995) in the male mice which were exposed to isoprene vapour for 26 weeks and then allowed to recover during an observation period of 26 weeks. Neurotoxicity was assessed by measuring forelimb and hindlimb grip strength at the end of the exposure period as well as on day 2 of the recovery period and after 1, 3 and 6 months of recovery. At the end of the 26-week exposure period, forelimb and hindlimb grip strength was significantly lower than in the controls at exposure levels of 220 ppm and above. During the recovery period, no significant differences were noted with respect to forelimb grip strength, whereas normal hindlimb grip strength was not reached until 3 months after the end of exposure. At the end of the observation period, the relative brain weights were significantly lower than those of the controls at concentrations of 700 ppm and higher. Histological examination at the end of the exposure period revealed that all of the animals which had been exposed to 7000 ppm and one mouse exposed to 2200 ppm had low-grade degenerative changes of the spinal cord white matter, and that 2 animals of the 7000 ppm group exhibited degenerative changes of the sciatic nerve. After 6 months of recovery, the incidence of low-severity spinal cord degeneration was significantly higher in all exposure groups than it was in the controls. Degeneration was characterised by dilated clear spaces in the white matter, with some of the spaces containing eosinophilic globules or “ovoids”. The spinal cord degeneration was considered by the authors to be the most likely cause of hindlimb dysfunction (Melnick et al., 1994, 1996; NTP, 1995).

In the mouse, the threshold level for reduced motor activity is approximately 1100 mg isoprene/m³ if the exposure period is 40 minutes (no further details; Gostinskii, 1965).

Cats were trained to produce a conditioned reflex (feeding reflex to a bell, light and buzzer). The latency phase between stimulus and movement reaction, and the speed of running up to be fed on hearing the signal served as the parameters of measurement.

Following a 1-hour exposure to 400 mg isoprene/m³, one cat displayed a slightly prolonged latency phase and reduction in the speed with which the

animals came running up to be fed. These changes persisted for 14 days. Two other cats showed more severe impairments (to the extent of failing to react to the stimulus) upon exposure to 500 and 700 mg/m³. The changes were reversible after 14 days. The threshold concentration was reported to be 400 mg isoprene/m³ (no further details; Korbakova and Fedorova, 1964).

Male rats (no indication of strain and number) were exposed to 4000 mg isoprene/m³ for 4 hours. After exposure, the “summation threshold value” and the escape reflex were investigated. Compared with a control group, there was a significant reduction in the “summation threshold value” and the number of attempts to escape (no further details; Repina, 1988).

7.11 Other effects

Haemoglobin adduct formation

In order to investigate whether isoprene forms adducts with haemoglobin, groups of 3 male B6C3F1 mice and 3 male Sprague-Dawley rats (12 to 14 weeks old) were given a single intraperitoneal injection of ^{14}C-labelled isoprene, dissolved in maize germ oil (corn oil), at doses of 0.3, 3, 300, 1000 or 3000 µmol/kg body weight (equivalent to approx. 0.02, 0.20, 20.4, 68.1 or 204.4 mg/kg body weight) or given intraperitoneal injections of 500 µmol/kg body weight (equivalent to approx. 34 mg/kg body weight) per day on 1, 2 or 3 days. Twenty-four hours after administration, the globin was isolated from the blood samples and the radioactivity was measured by liquid scintillation spectroscopy after appropriate sample work-up. Up to a dose of 500 µmol ^{14}C-labelled isoprene/kg body weight, the analytically determined amounts of ^{14}C-labelled adducts were linearly related to the administrated dose in both species. Repeated administration of 500 µmol/kg body weight showed that ^{14}C-labelled adducts accumulated in a virtually linear relationship to the administered dose. In both species, approximately the same amounts of ^{14}C-labelled adducts (approx. 40 pmol/mg globin at 3000 µmol/kg) were detected. The efficiency of adduct formation, corrected for the amount of isoprene eliminated by exhalation during the 24-hour period after injection (cf. Section 7.1), was 0.158 ± 0.035 and 0.079 ± 0.016 pmol ^{14}C-labelled adduct/mg globin)/(µmol retained ^{14}C-labelled isoprene/kg body weight) in mice and rats, respectively (Sun et al., 1989).

Male B6C3F1 mice (3 or 4 animals/group) were injected intraperitoneally with 33.2 mg unlabelled isoprene or 35.3 mg ^{14}C-labelled isoprene/kg body weight, dissolved in maize germ oil (corn oil), on days 1, 2, 3, 6 and 7 of the study. Blood samples were collected 24 hours after the last injection and analysed for haemoglobin adduct formation. At 24 hours after the last injection, 3 to 6% of the administered radioactivity was detected in the blood and 5% in the carcass. No details were given as to the quantities of haemoglobin adducts formed (NTP, 1994).

Groups of 4 male B6C3F1 mice (weighing approx. 28 g, 12 to 13 weeks old) inhaled 20, 200 or 2000 ppm ^{14}C-labelled isoprene (equivalent to approx. 56, 560 or 5600 mg/m³) for a single exposure period of 6 hours. Twenty-four hours after exposure, the haemoglobin was isolated and the radioactivity was measured following appropriate sample work-up. At the 20 ppm, 200 ppm and 2000 ppm exposure levels, haemoglobin adduct formation was 11 ± 0.5, 90 ± 13 and 170 ± 13 pmol/mg globin, respectively. Upon inhalation administration, larger amounts of haemoglobin adducts were formed at higher concentrations, which is in contrast to the findings described above for haemoglobin adduct formation upon intraperitoneal administration. The authors' explanation for this observation is that intraperitoneal injection represents a bolus dose resulting in higher systemic concentrations than those obtained with inhalation, and consequent saturation of isoprene metabolism (Bond et al., 1991).

Effects of isoprene on enzymes

Rabbits which were exposed to isoprene vapour (concentration not given) for 4 hours/day over 2 months showed a decrease in total proteins and an increase in the albumin-globulin ratio in the bone marrow (no further details; Faustov and Lobeeva, 1970).

Isoprene, dissolved in maize germ oil (corn oil), was administered (presumably orally), to 5 to 7 male Swiss mice at 500 mg/kg body weight for 2 days or 7 days consecutively in order to investigate the chemical's ability to modify the activity of enzymes catalysing the biotransformation of xenobiotics, and that of isoprene epoxidase. Compared with the controls, the liver microsomes of the isoprene-treated mice exhibited no changes in cytochrome b_5 and cytochrome P-450 concentrations or in aminopyrine N-demethylase, dinemorphan N-demethylase and isoprene epoxidase ac-

tivities. The investigations were carried out in two independent studies (no further details; Del Monte et al., 1985).

Endogenous formation of isoprene

In the mouse, guinea pig, rabbit, dog and chicken (no further details) no significant amounts of isoprene were detectable in the breath. In rats, isoprene was detected in the breath during lactation or when the animals were weaned to sour cream and cottage cheese (no further details; Gelmont et al., 1981).

Thin tissue slices (< 0.3 mm) from the livers and kidneys of pathogen-free rats were incubated in closed glass flasks for 3 hours at 37 °C in phosphate buffer and the overhead gas was subsequently analysed by gas chromatography. Small amounts of isoprene were detected. The quantities were greater when the tissues were obtained from younger rats 2 to 4 months old (no further details; Gelmont et al., 1981).

Endogenously formed isoprene was also detected in the blood of experimental animals. For these studies, blood was collected from 2 rats, 2 rabbits, 4 ponies, 5 dogs, 5 cows and 7 ewes and analysed by gas chromatography/mass spectrometry. In contrast to human blood (cf. Section 8), only very small quantities of isoprene (< 1 nmol/l) were detectable (no further details; Cailleux et al., 1992).

As measurable quantities of isoprene were only detected in the rat (see above; Gelmont et al., 1981), the attempt was made to demonstrate the in vitro biosynthesis of isoprene from DL-mevalonate, utilising the cytosolic fraction of rat liver. The cytosolic fraction was obtained from the livers of adult Sprague-Dawley rats (weighing 200 to 300 g). Aliquots of 3.5 ml of the fraction were incubated in reaction vials at 37 °C for 3 hours together with DL-mevalonate (10 mM) and adenosine triphosphate (5.0 mM). Detection of the isoprene formed during the reaction was accomplished by means of a head-space gas chromatography technique. Isoprene was detected in amounts of 418 ± 53 pmol. No isoprene was found in the incubations without DL-mevalonate. One incubation without adenosine triphosphate resulted in a 75-percent reduction of isoprene synthesis (108 ± 11 pmol). The formation of isoprene was increased 146-fold by acid treatment. In the authors' interpretation these results show that enzymatic conversion

of mevalonate leads to cytosolic intermediates of sterol biosynthesis, such as isopentenyl pyrophosphate and its isomer, dimethylallyl pyrophosphate, which apart from undergoing condensation to yield geranyl pyrophosphate, underwent nonenzymatic conversion to isoprene (Deneris et al., 1984, 1985).

8 Experience in humans

One woman and 2 men were each exposed for 5 minutes to isoprene concentrations ranging from 278 to 27800 mg/m³. The following results were seen: 278 mg/m³ odour just perceptible; 695 mg/m³ odour clearly perceptible; 2780 mg/m³ odour very clearly perceptible; 13900 mg/m³ odour very clearly perceptible, headache; 27800 mg/m³ clear bronchial irritation, severe headache (no further details; Bayer, 1972).

A concentration of 160 mg/m³ caused slight irritation of the mucous membranes of the nose, larynx and pharynx in 10 volunteers. The odour threshold was 10 mg/m³ (no further details; Gostinskii, 1965).

According to Muir (1971), liquid isoprene is also irritating to the skin and eyes.

Six volunteers (5 men, one woman) inhaled isoprene at concentrations of 0 (control), 8 and 50 ppm (equivalent to approx. 22 and 140 mg/m³) in a closed spirometer system (15.6 l including lung volume). The concentration-time courses of inhaled and exhaled isoprene were measured by gas chromatography up to 3 hours. Based on rat and mouse pharmacokinetic data (cf. Section 7.1; Peter et al., 1987), which were analysed using a two-compartment model that treats the system's gas phase and the body each as separate homogenous compartments with immediate and uniform substance distribution, concentration-time courses were predicted for humans. Curves extrapolated from the rat data were in better agreement with the measured human data than were those obtained with the mouse data. In the concentration range investigated, first-order kinetics were observed. Clearance was 81 l/hour. With the pulmonary ventilation rate being about 320 l/hour in these studies, pulmonary retention was found to be 25%. As was the case in rats and mice, accumulation in the human body was limited to about 3 times the atmospheric concentration of isoprene. The metabolism of inhaled isoprene was limited by transport to the metabolising sites rather than by metabolic capacity. The endogenous production rate was

calculated to be 0.15 µmol/(hour x kg body weight) (no further details; Hartmann and Kessler, 1990).

In addition, 6 subjects (5 men, one woman, aged between 19 and 41 years) were exposed to isoprene (purity: 99.5%) concentrations of 0, 8.3, 36.4, 42.2, 44.6 and 45.5 ppm (equivalent to 0, approx. 23, 102, 118, 124 and 127 mg/m³) in a closed exposure system (spirometer, volume 13.8 l) for up to 3 hours. The time course of the changes in concentration within the system after commencement of exposure was determined by gas chromatography. Pharmacokinetic analysis was accomplished by means of a two-compartment model (cf. above). The processes of inhalation uptake, exhalation and metabolism followed first-order kinetics. The production rate was regarded as being constant for the duration of the experiment. The pharmacokinetic data obtained in rats and mice (cf. Section 7.1; Peter et al., 1987) were extrapolated to each subject, employing a surface factor (body weight$^{2/3}$). These values and the endogenous production rate as the variable parameter were used to fit curves to the experimental data sets. Based on the parameters of the extrapolated curves, it was possible to calculate the following pharmacokinetic properties in humans. The body/ambient air concentration ratio remained virtually constant in the 10–50 ppm concentration range and, due to metabolism, was much lower than the thermodynamic body/air partition coefficient. Multiplication of the atmospheric concentration-based values for clearance of inhalation, clearance of exhalation and metabolic clearance with the atmospheric concentration yielded the inhaled, exhaled and metabolised quantities per unit of time at steady state. For isoprene concentrations in the 10–50 ppm range, the following values were extrapolated from the mouse and rat data, respectively: clearance of inhalation: 219 and 98 l/hour, clearance of exhalation: 70 and 14 l/hour and clearance of metabolism: 168 and 114 l/hour. According to the extrapolation results from mouse data, humans metabolise about 71% of the exogenous isoprene, the rest being exhaled unchanged. On the basis of rat data, isoprene uptake in humans was only half as much at the same ambient concentration, with 89% of the isoprene being metabolised and as little as 11% being exhaled unchanged. Alveolar retention of exogenous isoprene was 56% and 38% (extrapolated from the mouse and rat data, respectively), assuming an alveolar respiratory minute volume of 5 l. The endogenous production rates were 0.19 ± 0.04 µmol/hour/kg and 0.29 ± 0.06 µmol/hour/kg (extrapolated from mouse and

rat data, respectively). The average quantities of endogenous isoprene formed in the body was reported to be about 264 pmol/ml tissue and 332 pmol/ml tissue (extrapolated from mouse and rat data, respectively). The quantities of isoprene exhaled per day as calculated from these data were 6.0 mg and 3.8 mg (from mouse and rat data, respectively; Hartmann, 1994).

Groups of 4 and 5 healthy subjects (3 men, one woman, 54 to 75 kg, 19 to 34 years of age and 4 men, one woman, 54 to 91 kg, aged 28 to 41 years) were exposed to isoprene (purity: 99.5%) at concentrations of 0 and approximately 8 or 40 ppm (equivalent to approx. 22 or 112 mg/m³) in a closed spirometer system (volume: 13.8 or 12 l) with synthetic air (< 1% hydrocarbons) for 2.5 hours and the concentrations-time courses were determined by gas chromatography. At the 0 and 40 ppm exposure levels, endogenous production of isoprene resulted in plateau concentrations of approx. 0.6 ppm (approx. 1.7 mg/m³) at which the rate of metabolism equalled that of the endogenous formation. With the aid of a physiologically based toxicokinetic model, the endogenous production rate calculated for an individual of 70 kg was 23.8 µmol/hour and, based on this value, the amount of endogenously formed isoprene exhaled within 24 hours was computed to be 3.4 mg. This value is in agreement with the direct measurements (cf. e. g. Gelmont et al., 1981). The rate of metabolism of endogenously formed isoprene was given as 0.31 µmol/hour/kg body weight (no further details; in particular, no details of the results after exposure to 8 ppm; Filser et al., 1996).

In a study conducted in 12 healthy volunteers with no known medical problems, samples of inhaled air and breath were collected in the morning between 07.00 and 11.00 hours and analysed for volatile organic compounds by gas chromatography/mass spectrometry. The difference in concentrations in the breath and the inhaled air was used in order to calculate an alveolar gradient, which was found to be strongly negative for isoprene (approx. -2.1×10^5). According to the authors, a negative gradient indicates that a volatile organic compound originated from outside the body and was either catabolised or excreted via an extrapulmonary pathway (no further details; Phillips et al., 1994).

In 13 subjects, who were not working with the solvent, analysis by gas chromatography/mass spectrometry showed that their breath con-

tained an average isoprene concentration of 0.23 ppm (0.01 to 0.45 ppm; equivalent to 0.64 (0.03 to 1.26) mg/m³) (no details as to the collection period). No isoprene was detected in the expired air of up to 7-day-old newborn infants (Jansson and Larsson, 1969).

For each of 30 subjects, 30 l of breath were collected, a 3-l sample of which was analysed for hydrocarbon content after appropriate treatment. The isoprene concentration was 30 to 70% of total hydrocarbons exhaled. The exhaled quantity of isoprene was calculated to be between 2 and 4 mg per 24 hours. No differences were observed between the subjects with respect to age, sex, ethnic group, diet, style of living as well as fasting and non-fasting states. Each individual differed in the quantity of isoprene exhaled from day to day. In the authors' opinion, isoprene is an endogenous substance which is formed either as a by-product of isoprenoid biosynthesis or as an end product of isoprenoid degradation (Gelmont et al., 1981).

For 60 minutes, 8 male volunteers (aged 23 to 47 years, 5 nonsmokers, 3 smokers) inhaled air which contained no isoprene in amounts detectable by gas chromatography. In the subjects' expired air, which after appropriate preparation was also analysed by gas chromatography, 69 compounds were detected. The exhaled quantities of isoprene found in the smokers and nonsmokers ranged from 15 to 390 µg/hour and from 16 to 250 µg/hour, respectively. The largest amount of isoprene was found in one individual who felt he was not receiving sufficient air. The authors suggested that the production of isoprene may be associated with psychological stress or that it is the result of steroid-structured compound catabolism, or mevalonic acid synthesis (Conkle et al., 1975).

Exhaled air from 28 healthy volunteers representing an urban population (15 men, 13 women, ages ranging from 18 to 60 years, nonsmokers, no history of diseases or disorders, no medication) was collected 8 times for 3 to 4 minutes and, after appropriate preparatory steps, analysed by gas chromatography for organic constituents. Seven days prior and during sample collection, exposure to perfume, paint, glue, aerosols and dust, cigarette smoke as well as industrially polluted air was avoided. Background samples of the sample collection systems were analysed in 20 determinations over a period of 18 months. Evaluation by gas chromatography revealed 103 organic compounds of endogenous or exogenous origin in the exhaled air. Isoprene (33 ng/l) was found to be one of the major con-

stituents, in addition to acetone (120 ng/l) and acetonitrile (24 ng/l; Krotoszynski et al., 1977).

In a further study carried out by the same team of scientists, 387 samples of exhaled air collected from 54 healthy volunteers (35 men, 19 women) were analysed by means of the same method. 115 organic compounds of endogenous or exogenous origin were identified. In addition to isoprene, the major constituents proved to be dichloronitromethane, chloroform, 1-propanol, 2-methyl-1-butanol and dichloroacetylene (accounting for a total of 37.8% of the identified compounds). In 70% of the samples, isoprene was found in average concentrations of 28.9 ng/l. The authors referred to isoprene as a primary endogenous compound, the occurrence of which in exhaled air has been discussed as being associated with physiological stress and its origin with catabolism of CoQ and other steroid-structured compounds or mevalonic acid synthesis (Krotoszynski et al., 1979).

The concentration of isoprene in the exhaled air of 43 volunteers (20 men, 23 women, aged 22 to 75 years, nonsmokers) was determined in the morning, 3 hours after the last meal, by gas chromatography. For one hour prior to measurement, the subjects breathed room air which contained no detectable amounts of isoprene. The mean isoprene concentration in the exhaled air was 7.05 ± 3.53 nmol/l. No significant age-related change in isoprene concentration was found (Mendis et al., 1994).

Isoprene was determined in the expired air of 16 subjects (6 men, 10 women, nonsmokers) by means of gas chromatography and UV-spectrography. The subjects exhaled their breath, after holding it for 30 seconds, into a 5-l plastic collecting bag which was kept at a constant temperature of 35 °C. The mean isoprene concentration in the expired air was 3.73 ± 1.86 nmol/l (equivalent to 0.254 ± 0.127 µg/l). No sex-specific differences were observed. From 9 consecutive determinations carried out in one male subject over a period of 100 minutes, a mean concentration of 3.69 ± 0.60 nmol/l (equivalent to 0.251 ± 0.040 µg/ml) was calculated. The authors attributed the fact that breath isoprene values were lower than in other studies to the improved analytical procedure, which were certain to preclude other hydrocarbons, such as methane, ethane and n-pentane (Jones et al., 1995).

The concentration of isoprene in the alveolar air samples obtained from healthy subjects over a collection period of 6 to 8 hours was 28.3 ± 9.7 nmol/l, equivalent to approx. 1.93 ± 0.66 µg/l (25 samples). No differences were found between men and women. A diurnal rhythm was apparent, with isoprene levels decreasing during the day and increasing at night (no further details; DeMaster et al., 1976).

In a further study conducted in 50 volunteers (30 women and 20 men, aged 15 to 60 years) it was found that the concentration of endogenously formed isoprene in the breath depended on whether the individuals were awake or asleep. In the waking state, between 08.00 and 23.00 hours, the mean isoprene concentration determined for the subjects collectively was 14.6 ± 6.4 nmol/l (equivalent to 0.99 ± 0.44 µg/l). At night, 13 sleeping individuals exhaled concentrations in the range between 42.4 ± 13.5 and 45.3 ± 16.5 nmol/l (equivalent to 2.89 ± 0.92 and 3.09 ± 1.12 µg/l). On awakening, isoprene concentrations fell sharply to “waking values” within less than 20 minutes. Individuals (n = 9) who had remained awake during the night showed isoprene concentrations similar to those measured during the day (17.7 ± 7.0 nmol/l, equivalent to 1.21 ± 0.48 µg/l). Nine of the sleeping individuals also had isoprene levels monitored in the various stages of sleep (I, II, III, IV, REM). However, there was no apparent relationship between the measured isoprene levels and the stages of sleep (Cailleux and Allain, 1989).

Ten healthy subjects (9 men and one woman, mean age 26 ± 2 years, nonsmokers) underwent exposure to varying concentrations of ozone (initiated at 150 ppb, ramped stepwise to 350 ppb) or filtered air in a whole-body inhalation chamber for 130 minutes on different days. During the exposure periods, the subjects alternated between 10 minutes of rest and 10 minutes of treadmill exercise. Breath samples were collected spirometrically before and immediately after exposure, in the case of 6 individuals additionally at 19 ± 1 hours after the exposure, and the concentration of isoprene was determined by gas chromatography. The isoprene concentration (pre-exposure levels ranging from 0.37 to 3.2 nmol/l) was significantly decreased immediately post-exposure, independently of whether the subjects had been exposed to ozone or filtered air (on average by 19 and 17%, respectively). According to the authors, these results suggest that physical exercise alone reduces the isoprene concentration in breath without ozone adding to the effect. The 6 individuals who had their breath ana-

lysed 19 ± 1 hour after the exposure were found, particularly after ozone exposure, to exhale isoprene at concentrations which significantly exceeded the pre-exposure levels (enhancement over pre-exposure output was 99% with ozone and 51% with filtered air). The authors therefore suggested that isoprene concentration in exhaled breath could serve as a marker of a physiological response to oxidants, such as ozone, that cause injury to the epithelial membranes in the lower respiratory tract (Foster et al., 1996).

Endogenously formed isoprene was also detected by gas chromatography/mass spectrometry in blood obtained from 10 subjects (6 women and 4 men, aged 30 to 50 years). Blood samples of 10 ml were collected in the morning from the waking subjects. The concentrations of isoprene measured in human blood were between 15 and 70 nmol/l (equivalent to 1.02 and 4.77 µg/l), the mean value being 37 ± 25 nmol/l (equivalent to 2.52 ± 1.70 µg/l; Cailleux et al., 1992).

In order to investigate the connection between the formation of endogenous isoprene, which is detected in breath, and cholesterol biosynthesis (cf. Section 7.11; Deneris et al., 1984, 1985) in humans, volunteers were treated with lovastatin, a drug that inhibits cholesterol biosynthesis, either in single doses or over a period of 6 weeks, or they were kept on a cholesterol-enriched diet for 6 weeks. In the single-dose studies, 20, 40 or 80 mg lovastatin were administered at 20.00 hours to 5 healthy male subjects (aged 18 to 50 years). Breath isoprene concentration was determined by gas chromatography at 4-hour intervals 24 hours before and after dosing. Compared with the isoprene levels prior to lovastatin administration, all three doses significantly reduced breath isoprene concentrations (by ≥ 36%) at 6 and 10 hours after administration. The 6-week daily administration of 40 mg lovastatin to 8 male subjects (aged 30 to 72 years) reduced breath isoprene levels by 27 ± 9% and cholesterol synthesis in freshly isolated mononuclear leucocytes (measured via the incorporation of ^{14}C-labelled acetate into sterols) by 12 ± 6%. In the 8 subjects who followed a cholesterol-enriched diet (1070 mg/day), breath isoprene concentration and cholesterol synthesis in mononuclear leucocytes were reduced by 16 ± 5 and 19 ± 4%, respectively. The values were obtained in the mornings between 06.00 and 07.00 hours before and after the 6-week treatment. According to the authors, the parallel decrease in isoprene concentration and cholesterol synthesis in mononuclear leucocytes suggests

that breath isoprene is derived from the cholesterol synthesis pathway (Stone et al., 1993).

There are reports of isoprene affecting various enzymes in lymphocytes/granulocytes (suppression of succinate dehydrogenase activity, increase in alkaline and acid phosphatase activity) in workers in the rubber industry. The workers were exposed to isoprene and, in addition, to styrene, butadiene, isobutylene and chloromethane (no further details; Mamedov and Aliev, 1985 a, b). The enzymes under assessment are subject to high variability (alkaline and acid phosphatase) or great influence by hormones (succinate dehydrogenase). Variability, mixed exposure and inadequate documentation of methods and results render these studies unsuitable for assessment of the effects of isoprene in humans.

In 630 employees (350 men, 280 women) who worked in isoprene rubber production and were exposed to formaldehyde, dimethyldioxane and isoprene at concentrations that corresponded to, or were less than, the Soviet MAC values (0.5, 10 and 40 mg/m³, respectively), had their upper respiratory tracts examined over a period of 4 years (from 1965 to 1968). A large percentage of pathological changes was found, which increased with the duration of employment. Individuals employed in this area of production for one year or less exhibited catarrhal inflammation of the nose as the predominant change. In employment of longer duration, there was an increase in the amount of degenerative and atrophic processes. The temperature of the nasal mucosa was lower in workers employed in the production of synthetic rubber than it was in workers of a control group (32.4 ± 0.1 °C, control group 33.1 ± 0.4 °C). The pH of nasal discharge was slightly more acidic (7.0, control group 7.2). Nasal mucosa cytograms showed signs of inflammatory and degenerative changes in the nasal mucosa. The incidence and degree of olfactory deterioration also increased with the duration of employment (Mitin, 1969). As the study investigated mixed exposure in the workplace and did not give details of experimental results, it is not suitable for assessment of the effects of isoprene in humans.

9 Classifications and threshold limit values

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") of the Deut-

sche Forschungsgemeinschaft will investigate the possibility of establishing a MAK value for the chemical as well as the necessity for classification of its carcinogenic potential (DFG, 2000).

The former USSR defined a “Short Term Exposure Limit” of 40 mg/m³ air (Shugaev, 1969; Taalman, 1996; IARC, 1994), and Poland and Bulgaria set TLV values of 100 and 10 mg/m³, respectively (IARC, 1994; Taalman, 1996).

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