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Concise International Chemical Assessment Document 5

LIMONENE

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The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

Concise International Chemical Assessment Documents (CICADs) are the latest in a family of publications from the International Programme on Chemical Safety (IPCS) — a cooperative programme of the World Health Organization (WHO), the International Labour Organisation (ILO), and the United Nations Environment Programme (UNEP). CICADs join the Environmental Health Criteria documents (EHCs) as authoritative documents on the risk assessment of chemicals.

CICADs are concise documents that provide summaries of the relevant scientific information concerning the potential effects of chemicals upon human health and/or the environment. They are based on selected national or regional evaluation documents or on existing EHCs. Before acceptance for publication as CICADs by IPCS, these documents have undergone extensive peer review by internationally selected experts to ensure their completeness, accuracy in the way in which the original data are represented, and the validity of the conclusions drawn.

The primary objective of CICADs is characterization of hazard and dose–response from exposure to a chemical. CICADs are not a summary of all available data on a particular chemical; rather, they include only that information considered critical for characterization of the risk posed by the chemical. The critical studies are, however, presented in sufficient detail to support the conclusions drawn. For additional information, the reader should consult the identified source documents upon which the CICAD has been based.

Risks to human health and the environment will vary considerably depending upon the type and extent of exposure. Responsible authorities are strongly encouraged to characterize risk on the basis of locally measured or predicted exposure scenarios. To assist the reader, examples of exposure estimation and risk characterization are provided in CICADs, whenever possible. These examples cannot be considered as representing all possible exposure situations, but are provided as guidance only. The reader is referred to EHC 170¹ for advice on the derivation of health-based guidance values.

While every effort is made to ensure that CICADs represent the current status of knowledge, new information is being developed constantly. Unless otherwise

stated, CICADs are based on a search of the scientific literature to the date shown in the executive summary. In the event that a reader becomes aware of new information that would change the conclusions drawn in a CICAD, the reader is requested to contact the IPCS to inform it of the new information.

Procedures

The flow chart shows the procedures followed to produce a CICAD. These procedures are designed to take advantage of the expertise that exists around the world — expertise that is required to produce the high-quality evaluations of toxicological, exposure, and other data that are necessary for assessing risks to human health and/or the environment.

The first draft is based on an existing national, regional, or international review. Authors of the first draft are usually, but not necessarily, from the institution that developed the original review. A standard outline has been developed to encourage consistency in form. The first draft undergoes primary review by IPCS and one or more experienced authors of criteria documents to ensure that it meets the specified criteria for CICADs.

The second stage involves international peer review by scientists known for their particular expertise and by scientists selected from an international roster compiled by IPCS through recommendations from IPCS national Contact Points and from IPCS Participating Institutions. Adequate time is allowed for the selected experts to undertake a thorough review. Authors are required to take reviewers' comments into account and revise their draft, if necessary. The resulting second draft is submitted to a Final Review Board together with the reviewers' comments.

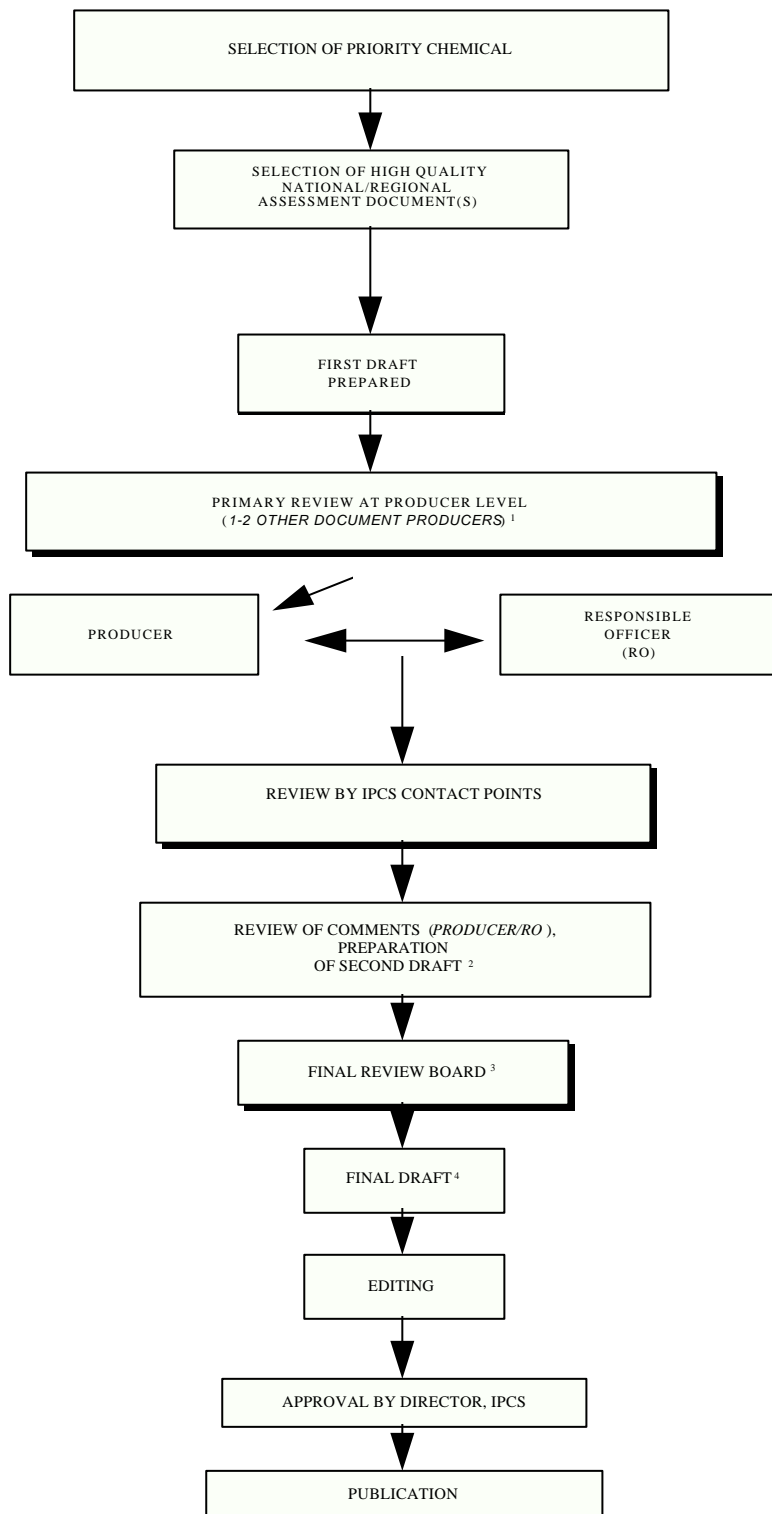
The CICAD Final Review Board has several important functions:

- to ensure that each CICAD has been subjected to an appropriate and thorough peer review;
- to verify that the peer reviewers' comments have been addressed appropriately;
- to provide guidance to those responsible for the preparation of CICADs on how to resolve any remaining issues if, in the opinion of the Board, the author has not adequately addressed all comments of the reviewers; and
- to approve CICADs as international assessments.

Board members serve in their personal capacity, not as representatives of any organization, government, or industry. They are selected because of their expertise in human and environmental toxicology or because of their experience in the regulation of chemicals. Boards are chosen according to the range of expertise required for

¹ International Programme on Chemical Safety (1994) *Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits*. Geneva, World Health Organization (Environmental Health Criteria 170).

CICAD PREPARATION FLOW CHART



1 Revision as necessary.

2 Taking into account the comments from reviewers.

3 The second draft of documents is submitted to the Final Review Board together with the reviewers' comments (6-10 CICADs are usually reviewed at the Final Review Board). In the case of pesticides the role of the Final Review Board is fulfilled by a joint meeting on pesticides.

4 Includes any revisions requested by the Final Review Board.

a meeting and the need for balanced geographic representation.

Board members, authors, reviewers, consultants, and advisers who participate in the preparation of a CICAD are required to declare any real or potential conflict of interest in relation to the subjects under discussion at any stage of the process. Representatives of nongovernmental organizations may be invited to observe the proceedings of the Final Review Board. Observers may participate in Board discussions only at the invitation of the Chairperson, and they may not participate in the final decision-making process.

1. EXECUTIVE SUMMARY

This CICAD on limonene (*d*-limonene, *l*-limonene, and *d/l*-limonene) was based primarily on a review prepared in 1993 for the Nordic Expert Group (Karlberg & Lindell, 1993). A second review produced under the auspices of the Nordic Council of Ministers (Josefsson, 1993), a preliminary, non-peer-reviewed information source on environmental exposure and effects (US EPA, 1994), and searches of relevant databases covering the years 1993–1995 were used for the identification of additional data for the assessment of limonene. In a final search of the literature from 1996 to 1997, no data that would change the conclusions made in the CICAD were identified. Information concerning the nature and availability of the source document is presented in Appendix 1. Information on the peer review of this CICAD is presented in Appendix 2. This CICAD was approved for publication at a meeting of the Final Review Board, held in Brussels, Belgium, on 18–20 November 1996. Participants at the Final Review Board meeting are listed in Appendix 3. The International Chemical Safety Card (ICSC 0918) for *d*-limonene, produced by the International Programme on Chemical Safety (IPCS, 1993), has also been reproduced in this document. Emphasis was given to *d*-limonene owing to the large amount of available data on this isomeric form.

Limonene occurs naturally in certain trees and bushes. Limonene and other monoterpenes are released in large amounts mainly to the atmosphere, from both biogenic and anthropogenic sources. Limonene is used as a solvent in degreasing metals prior to industrial painting, for cleaning in the electronic and printing industries, and in paint as a solvent. Limonene is also used as a flavour and fragrance additive in food, household cleaning products, and perfumes.

Limonene is a skin irritant in both experimental animals and humans. In rabbits, *d*-limonene was found to be an eye irritant. Studies in guinea-pigs revealed that air-oxidized *d*-limonene, but not *d*-limonene itself, induced contact allergy. Because *d*- and *l*-limonene are enantiomers, this could also be true for *l*-limonene and dipentene (the mixture). Handling and purity of the chemical, and possibly addition of antioxidants, may thus be crucial for the allergenic capacity of limonene.

The critical organ in animals (except for male rats), following peroral or intraperitoneal administration, is the liver. Studies in which experimental animals were exposed by inhalation to limonene have not been identified. Exposure to limonene affects the amount and activity of different liver enzymes, liver weight, cholesterol levels, and bile flow. These changes have been observed in mice, rats, and dogs. Available data are insufficient to determine the critical organ in humans.

In male rats, exposure to *d*-limonene causes damage to the kidneys and renal tumours. The male rat specific protein "2: -globulin is considered to play a crucial role in the development of neoplastic as well as non-neoplastic kidney lesions. Thus, these kidney lesions are considered not relevant for human risk assessment. *d*-Limonene has been studied in a battery of short-term *in vitro* tests and found to be non-genotoxic. There is no evidence that limonene has teratogenic or embryotoxic effects in the absence of maternal toxicity. In general, *d*-limonene could be considered (with the exception of its irritative and sensitizing properties) to be a chemical with fairly low toxicity.

Food is the principal source of exposure to limonene, based on available data. A guidance value for the ingestion of limonene was calculated to be 0.1 mg/kg body weight per day. At current estimated levels of exposure, limonene in foodstuffs does not appear to represent a significant risk to human health.

In the atmosphere, limonene and other terpenes react rapidly with photochemically produced hydroxyl and nitrate radicals and ozone. The oxidation of terpenes such as limonene contributes to aerosol and photochemical smog formation. In soil, limonene is expected to have low mobility; in the aquatic environment, it is expected to bind strongly to sediment. Limonene is resistant to hydrolysis. Biodegradation occurs under aerobic, but not anaerobic, conditions.

Terrestrial organisms are most likely exposed to limonene via the air. The few studies on terrestrial species (i.e. insects) using vapour exposure revealed effects of limonene at parts per million levels. Measured environmental concentrations are typically around 0.1–2 ppb (0.6–11 : g/m³). At polluted sites, limonene concentrations in soil may exceed effect levels of soil-living organisms (e.g. earthworms). In the aquatic environment, limonene shows high acute toxicity to fish and *Daphnia*. Limonene concentrations in surface waters are generally much lower than experimentally determined acute toxicity levels, and therefore it is likely that limonene poses a low risk for acute toxic effects on aquatic organisms. No studies were found on chronic effects.

2. IDENTITY AND PHYSICAL/CHEMICAL PROPERTIES

Limonene is a colourless liquid at room temperature. The structural formula for limonene is given below. The chemical exists as two optical isomers, *d*- and *l*-limonene, and the racemic mixture dipentene. The purity of commercial *d*-limonene is about 90–98%.

Table 1: Physical/chemical properties of limonene.^a

	<i>d</i> -Limonene	<i>l</i> -Limonene	Dipentene
CAS no.	5989-27-5	5989-54-8	138-86-3
Chemical name	(<i>R</i>)-1-methyl-4-(1-methylethenyl) cyclohexene	(<i>S</i>)-1-methyl-4-(1-methylethenyl) cyclohexene	1-methyl-4-(1-methylethenyl) cyclohexene
Empirical formula	C ₁₀ H ₁₆	C ₁₀ H ₁₆	C ₁₀ H ₁₆
Molecular weight	136.23	136.23	136.23
Melting point (°C)	! 74.35	! 74.35	! 95.9
Boiling point (°C)	175.5–176.0	175.5–176.0	175.5–176.0
Density (g/cm ³ at 20°C)	0.8411	0.8422	0.8402
Vapour pressure (Pa at 20°C)	190	190	190
Water solubility (mg/litre at 25°C)	13.8 ^b	–	–
Henry's law constant (kPa m ³ /mol at 25°C)	34.8 ^c	–	–
Log <i>K</i> _{ow}	4.23 ^d	–	4.83 ^e (limonene)

^a Conversion factors: 1 ppm = 5.56 mg/m³; 1 mg/m³ = 0.177 ppm.

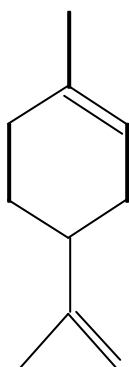
^b Massaldi & King, 1973; Assessment Tool for the Evaluation of Risk (ASTER) database, Environmental Research Laboratory, US Environmental Protection Agency, Duluth, MN, 1991.

^c Calculated value (ENVIROFATE database, Office of Toxic Substances, US Environmental Protection Agency, and Syracuse Research Corporation [SRC], New York, NY, 1995).

^d Calculated value (US EPA, 1990a, 1994).

^e Calculated value (US EPA, 1994; Log Octanol–Water Partition Coefficient Program [LOGKOW], Syracuse Research Corporation [SRC], New York, NY).

Physical and chemical data on limonene presented in Table 1 were taken from Karlberg & Lindell (1993), unless otherwise stated. Impurities are mainly other monoterpenes, such as myrcene (7-methyl-3-methylene-1,6-octadiene), α -pinene (2,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene), β -pinene (6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane), sabinene (2-methyl-5-(1-methylethyl)-bicyclo[3.1.0]hexan-2-ol), and γ -carene ((1*S*-cis)-3,7,7-trimethyl-bicyclo[4.1.0]hept-2-ene). The vapour pressure of limonene is high and its solubility in water is low, giving a high value of the Henry's law constant, which predicts a high rate of vaporization of limonene.



3. ANALYTICAL METHODS

Airborne limonene may be collected by charcoal tube sampling followed by desorption with carbon disulfide (Searle, 1989) or alternatively on Tenax (Janson &

Kristensson, 1991) or on multisorbent sampling tubes (Chan et al., 1990) followed by thermal desorption. Limonene is usually analysed by gas chromatography with flame ionization detection or mass spectrometry. For limonene in blood, liquids, and tissues, a head-space technique could be used. The detection limit in air is 5 : g/m³ (Searle, 1989) and in blood, 1.4 : g/litre (Falk Filipsson et al., 1993). As limonene is easily oxidized in air, it is also important to analyse the oxidation products. Hydroperoxides of *d*-limonene can be analysed by gas chromatography if the sample is injected on-column (Karlberg et al., 1994). A high-performance liquid chromatography method for limonene has also been developed (Nilsson et al., 1996).

4. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

Limonene, like other monoterpenes, occurs naturally in certain trees and bushes. It is found in peel from citrus fruits, in dill, caraway, fennel, and celery, and in turpentine. Typical concentrations of monoterpenes in air in conifer forests are 1–10 : g/m³, but variations are large (Strömvall, 1992). Mean emission rates of limonene from different plant species (i.e. lemon, orange, pistachio, and walnut) in the Central Valley of California ranged from 0.4 to 2.5 mg/g dry leaf weight per hour (Arey et al., 1991). Monoterpenes are released in significant amounts mainly to the atmosphere. Biogenic emissions are in the order of, or may exceed, those from anthropogenic sources (Dimitriadis, 1981; Altshuler, 1983; Lamb et al., 1987). Global annual emissions of biogenic

monoterpenes range from 147 to 827 million tonnes (Fehsenfeld et al., 1992).

Limonene is used as a substitute for chlorinated hydrocarbons, chlorofluorocarbons, and other solvents. It is used in degreasing metals (30% limonene) prior to industrial painting, for cleaning in the electronic industry (50–100% limonene), for cleaning in the printing industry (30–100% limonene), and in paint as a solvent. Limonene is also used as a solvent in histological laboratories and as a flavour and fragrance additive in food, household cleaning products, and perfumes. *d*-Limonene has been used as a gallstone solubilizer in humans (Igimi et al., 1976, 1991).

The annual worldwide production of *d*-limonene and orange oil/essence oil (95% *d*-limonene) in 1991 was approximately 45 kt (Florida Chemical Co., 1991). Citrus plantings under way are expected to increase that figure to 73 kt annually within a decade (IARC, 1993). Production volumes in Japan were about 40 kt in each of 1992 and 1993 (Chemical Daily, 1994, 1995). In 1984, the US consumption of *d*-limonene was 250 t.¹ The number of industrial plants in the USA handling *d*-limonene in 1983 was 87, and the estimated number of employees exposed to the chemical was 140 000.² The corresponding numbers of industrial plants and exposed employees were 2 and 1843 for *l*-limonene and 103 and 185 000 for dipentene, respectively. In 1974, the corresponding numbers for dipentene were 70 and 45 000, respectively. The increased use of dipentene has probably continued after 1983, especially because of its use as a substitute for chlorinated hydrocarbons, chlorofluorocarbons, and other solvents, but no production data were identified. According to the product register set up by the Swedish National Chemicals Inspectorate, between 69 and 80 t of *d*-limonene in 48 products (15 for consumers) were used during 1994 in Sweden. The corresponding numbers for dipentene were 74–88 t in 106 products (26 for consumers). No use of *l*-limonene was reported.

¹ Source: Environmental Chemicals Data and Information Network (ECDIN). Ispra, Italy, CEC Joint Research Centre (1993).

² Source: Registry of Toxic Effects of Chemical Substances (RTECS). US Department of Health and Human Services, National Institute of Occupational Safety and Health (NIOSH) (1994).

5. ENVIRONMENTAL TRANSPORT, DISTRIBUTION, AND TRANSFORMATION

Monoterpenes such as limonene are released in large amounts mainly to the atmosphere. The chemical and physical properties of limonene also indicate that the substance will be distributed mainly to air.

When released to ground, limonene is expected to have low to very low mobility in soil, based on its physical/chemical properties. The soil adsorption coefficient (K_{oc}), calculated on the basis of the solubility (13.8 mg/litre at 25°C) and the log octanol/water partition coefficient (4.232), ranges from 1030 to 4780.³ The Henry's law constant indicates that limonene will rapidly volatilize from both dry and moist soil; however, its strong adsorption to soil may slow this process.³

In the aquatic environment, limonene is expected to adsorb to sediment and suspended organic matter and to rapidly volatilize to the atmosphere, based on its physical/chemical properties.³ The estimated half-life for volatilization of limonene from a model river (1 m deep, flow 1 m/s, and wind speed 3 m/s) is 3.4 hours.³ The bioconcentration factor, calculated on the basis of water solubility and the log octanol/water partition coefficient, is 246–262,³ suggesting that limonene may bioaccumulate in fish and other aquatic organisms.

Limonene does not have functional groups for hydrolysis, and its cyclohexene ring and ethylene group are known to be resistant to hydrolysis (US EPA, 1994). Therefore, hydrolysis of limonene is not expected, neither in terrestrial nor in aquatic environments. The hydrolytic half-life of *d*-limonene has been estimated to be >1000 days.⁴ Biotic degradation of limonene has been shown with some species of microorganisms, such as *Penicillium digitatum*, *Corynespora cassiicola*, *Diplodia gossypina* (Abraham et al., 1985), and a soil strain of *Pseudomonas* sp. (PL strain) (Dhavalikar & Bhattacharayya, 1966; Shulka & Bhattacharayya, 1968). As these studies were not designed to determine the biodegradability of limonene, the results provided only indications of possible biodegradation. However, limonene was readily biodegradable (41–98% degradation by biochemical oxygen demand in 14 days) under aerobic conditions in a standard test (OECD 301 C "Modified MITI Test (I)"; OECD, 1981) (MITI, 1992). Also, in a test simulating aerobic sewage treatment (OECD 303 A

³ Source: Hazardous Substances Data Bank. Bethesda, MD, National Library of Medicine (1995).

⁴ Source: ASTER (Assessment Tool for the Evaluation of Risk) database. Duluth, MN, US Environmental Protection Agency, Environmental Research Laboratory.

Table 2: Rate constants and lifetimes of *d*-limonene in gas-phase reactions with hydroxyl radicals (OH), ozone (O₃), and nitrate radicals (NO₃).

Substance	Concentration (molecules/cm ³) ^a	Lifetime (hours)	Rate constant (cm ³ molecule ⁻¹ s ⁻¹)	Reference
OH	1×10 ⁶ (0.04 ppt)	0.32	9.0×10 ⁻¹⁰	Winer et al., 1976
	4×10 ⁶ (0.16 ppt)	0.5	1.4×10 ⁻¹⁰	Atkinson et al., 1984; Winer et al., 1984
	1×10 ⁶ (0.04 ppt)	1.6	1.7×10 ⁻¹⁰	Atkinson, 1990
	1×10 ⁶ (0.04 ppt)	2	1.4×10 ⁻¹⁰	Atkinson & Carter, 1984
	1×10 ⁶ (0.04 ppt)	2	1.4×10 ⁻¹⁰	Atkinson et al., 1984; Winer et al., 1984
O ₃	200 ppb	0.18	6.4×10 ⁻¹⁶	Atkinson et al., 1984; Winer et al., 1984
	7×10 ¹¹	0.5 ^b	5.4×10 ⁻¹⁶	Klöpffer et al., 1988
	30 ppb	0.6	6.4×10 ⁻¹⁶	Atkinson et al., 1984; Winer et al., 1984
	7×10 ¹¹	0.62	6.4×10 ⁻¹⁶	Atkinson, 1990
	7×10 ¹¹	0.67	6.0×10 ⁻¹⁶	Atkinson & Carter, 1984
	7×10 ¹¹	1.9	2.09×10 ⁻¹⁶	Atkinson et al., 1990
NO ₃	100 ppt	0.015 (0.9 min)	7.7×10 ⁻¹²	Atkinson et al., 1984; Winer et al., 1984
	2.4×10 ⁸	0.08 (5 min)	1.4×10 ⁻¹¹	Atkinson & Carter, 1984
	2.4×10 ⁸	0.09 (5.3 min)	1.3×10 ⁻¹¹	Atkinson, 1990
	10 ppt	0.15 (9 min)	7.7×10 ⁻¹²	Atkinson et al., 1984; Winer et al., 1984

^a Unless otherwise indicated.

^b Half-life (in hours).

“Simulation Test — Aerobic Sewage Treatment: Coupled Units Test”; OECD, 1981), limonene disappeared almost completely (>93.8%) during 14 days of incubation (Schwartz et al., 1990). However, this test was not suitable for such a volatile substance as limonene. The disappearance of limonene was likely due in part to volatilization, but it could not be determined to what extent the removal was due to biodegradation and sorption compared with volatilization.

Biodegradation has also been assessed under anaerobic conditions. In a test on methanogenic degradation (batch bioassay inoculated with granular sludge, 30°C), there was no indication of any metabolism of limonene, possibly because of toxicity to the microorganisms (Sierra-Alvarez et al., 1990). Complex chlorinated terpenes, similar to toxaphene (a persistent, mobile, and toxic insecticide, with global distribution) and its degradation products, were produced by photo-initiated reactions in an aqueous system initially containing limonene and other monoterpenes, simulating pulp bleaching conditions (Larson & Marley, 1988).

In the atmosphere, limonene is expected to rapidly undergo gas-phase reactions with photochemically produced hydroxyl radicals, ozone, and nitrate radicals (Table 2). Based on experimentally determined rate constants, calculated lifetimes for the reaction of *d*-limonene with photochemically produced hydroxyl radicals range from 0.3 to 2 hours (Winer et al., 1976, 1984; Atkinson & Carter, 1984; Atkinson et al., 1984;

Atkinson, 1990). The corresponding lifetimes for the reaction with ozone are in the range of 0.2–2.6 hours (Atkinson & Carter, 1984; Atkinson et al., 1984, 1990; Winer et al., 1984; Klöpffer et al., 1988; Nolting & Zetzsch, 1988; Atkinson, 1990). Based on experimentally determined rate constants, calculated lifetimes for the nighttime reaction of *d*-limonene with nitrate radicals range from 0.9 to 9 minutes (Atkinson & Carter, 1984; Atkinson et al., 1984; Winer et al., 1984; Atkinson, 1990). The daytime atmospheric lifetime of *d*-limonene has been estimated to range from 12 to 48 minutes, depending upon the local hydroxyl radical and ozone concentrations (Altshuller, 1983).

Products formed from the hydroxyl radical reaction with limonene are 4-acetyl-1-methylcyclohexene (Arey et al., 1990; Grosjean et al., 1992; Hakola et al., 1994), a keto-aldehyde (Arey et al., 1990; Hakola et al., 1994), formaldehyde, 3-oxobutanal, glyoxal, and a C₁₀ dicarbonyl (Grosjean et al., 1992). The same carbonyls, along with formic acid and C₈ and C₉ carboxylic acids, may also form in reactions with ozone (Grosjean et al., 1992). Ozonolysis of limonene may also result in bis(hydroxymethyl)peroxide, a precursor to hydroxymethyl hydroperoxide (Gäb et al., 1985), and hydrogen peroxide (Becker et al., 1990). Hydroxymethyl hydroperoxide, bis(hydroxymethyl)peroxide, and hydrogen peroxide have various toxic effects on plant cells and enzymes (Gäb et al., 1985; Becker et al., 1990). The reaction of *d*-limonene with ozone in the dark results in the formation of 4-acetyl-1-methylcyclohexene and

formaldehyde (Grosjean et al., 1993). Reactions with nitrogen oxides produce aerosol formation as well as lower molecular weight products, such as formaldehyde, acetaldehyde, formic acid, acetone, and peroxyacetyl nitrate (Altshuller, 1983).

Terpenes such as limonene contribute to aerosol and photochemical smog formation (Gäb et al., 1985; Sekiya et al., 1988). Emissions of biogenic hydrocarbons such as limonene and other terpenes to the atmosphere may either decrease ozone concentrations when nitrogen oxide concentrations are low or, if emissions take place in polluted air (i.e. containing high nitrogen oxide levels), lead to an increase in ozone concentrations (Altshuller, 1983; Fehsenfeld et al., 1992).

6. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

6.1 Environmental levels

Data on environmental levels of limonene are presented in Table 3. The concentrations of limonene and other monoterpenes in air vary considerably. Recorded concentrations in rural areas depend on many factors, such as the type of vegetation, temperature, time of the day, and time of the year (Strömvall, 1992). Biogenic monoterpene emissions are assumed to be very low in the late autumn and winter months compared with summer (Altshuller, 1983). Measured concentrations (between 1979 and 1992) of limonene in the air of rural forest areas in Europe, Canada, the USA, Nepal, the Republic of Georgia, and Japan ranged from 1.6×10^{-4} to 2.2 ppb (0.9 ng/m³ to 12.2 : g/m³) (Shaw et al., 1983; Hutte et al., 1984; Roberts et al., 1985; Jüttner, 1986, 1988; Petersson, 1988; Helmig et al., 1989; Clement et al., 1990; Janson & Kristensson, 1991; Ciccoioli et al., 1992, 1993; Helmig & Arey, 1992; Peters et al., 1994). Based upon these data, typical concentrations of limonene in air from rural areas range from 0.1 to 0.2 ppb (0.6–1.1 : g/m³).

On the basis of measured concentrations (between 1973 and 1990) of limonene in the air from urban or suburban areas in Europe, the USA, and Russia that ranged from not detectable to 5.7 ppb (31.7 : g/m³) (Bertsch et al., 1974; Ioffe et al., 1977, 1979; Hutte et al., 1984; De Bortoli et al., 1986; Jüttner, 1988; Ciccoioli et al., 1992; Helmig & Arey, 1992), typical concentrations of limonene in urban/suburban air are likely to range from 0.1 to 2 ppb (0.6–11.1 : g/m³). Concentrations of limonene in air emissions from kraft pulp industries, stone groundwood production, and various waste and landfill sites have ranged from approximately 0.3 to

41 000 ppb (1.7 : g/m³ to 240 mg/m³) (Young & Parker, 1983, 1984; Koe & Ng, 1987; Strömvall, 1992; Eitzer, 1995).

Limonene has been detected in groundwater and surface waters, ice, sediments, and soil. Mean limonene concentrations in two polluted Spanish rivers were 590 and 1600 ng/litre (Gomez-Belinchon et al., 1991). Samples of water collected from the Gulf of Mexico contained limonene at a concentration of 2–40 ng/litre (Sauer, 1981). Limonene has also been detected at Terra Nova Bay, Antarctica; water and pack ice samples contained limonene at concentrations up to 20 and 15 ng/litre, respectively (Desideri et al., 1991). Limonene concentrations up to 920 : g/g in soil and from 1 to 130 : g/litre in groundwater were measured in a polluted area at a former site for the production of charcoal and pine tar products in Florida (McCreary et al., 1983). Limonene was also detected but not quantified in fish (i.e. carp) collected from Las Vegas Wash, Nevada (Hiatt, 1983).

6.2 Human exposure

Examples of estimated exposure to limonene in the general and occupational environments are presented here, on the basis of identified data primarily from the USA and Sweden. Countries are strongly encouraged, however, to estimate exposure on the basis of local data, possibly in a manner similar to that outlined here.

The intake in food may be unavoidable, as limonene occurs naturally in citrus fruits and spices and is used as a flavour and fragrance additive. However, there is a considerable interindividual variation in intake, owing to different diet patterns. Based on daily US consumption of *d*-limonene per capita, the intake of *d*-limonene from food for the general population was estimated to be 0.27 mg/kg body weight per day (Flavor and Extract Manufacturers Association, 1991).

Indoor concentrations of limonene (no specification of enantiomer) in northern Italy ranged from 10 to 480 : g/m³ (mean 140 : g/m³) (De Bortoli et al., 1986), whereas concentrations ranged from 1.6 to 78 : g/m³ (mean 18 : g/m³) in 17 residences in Ruston, Washington (Montgomery & Kalman, 1989). In an investigation from Los Angeles, California, the arithmetic mean limonene level in indoor air was 40 : g/m³ (Wallace et al., 1991). In 754 randomly selected residences in Canada, indoor concentrations of limonene ranged from 9 to 30 : g/m³ (Fellin & Otson, 1993); concentrations were higher during the winter season when ventilation was lower.

The intake of limonene from indoor and outdoor air for the general population is estimated to be 10 and 0.1 : g/kg body weight per day, respectively. This is

Table 3: Concentrations of limonene in various media.

Medium	Concentration	Location and sampling date	Reference
Air, rural	0.036 : g/m ³ (6.4×10 ⁻³ ppb)	Whitaker's Forest, Sierra Nevada Mountains, California, June 1990	Helmig & Arey, 1992
	0.49 ng/litre (8.7×10 ⁻² ppb)	Monte Cimini, Italy, (forest site)	Ciccoioli et al., 1992
	detected	Esgegebirge, North Rhine-Westfalia, Germany, 1988 (forest site)	Helmig et al., 1989
	40 ppbC ^a (25 : g/m ³) ^a	Forest site in Republic of Georgia, July 1979	Shaw et al., 1983
	0.030 ppb	Rocky Mountains, Colorado, average day July–Dec. 1982	Roberts et al., 1985
	0.072 ppb	Rocky Mountains, Colorado, average night July–Dec. 1982	Roberts et al., 1985
	0.002–0.13 ppb	Rocky Mountains, Colorado, range night July–Dec. 1982	Roberts et al., 1985
	detected	Western Colorado	Hutte et al., 1984
	0.34 : g/m ³ (6.0×10 ⁻² ppb)	Eastern Germany, July (forest site)	Ciccoioli et al., 1993
	1.16 : g/m ³ (0.20 ppb)	Nepal, September–October, 1991	Ciccoioli et al., 1993
	1.3–7.3 : g/m ³ (0.23–1.3 ppb)	Forest, Jönköping, Sweden, night June–July, 1983	Petersson, 1988
	0.1–2.2 ppb (0.6–12.2 : g/m ³)	Forest, Northwest Quebec, Canada, July 1989	Clement et al., 1990
	detected	Southern Black Forest, Germany, Nov.–Jan. (1984–1985)	Jüttner, 1986
	0.9–89 ng/m ³ (1.6×10 ⁻⁴ – 1.6×10 ⁻² ppb)	Southern Black Forest, Germany, March–Dec. 1985	Jüttner, 1988
	<0.05–0.25 ng/litre (<8.8×10 ⁻³ – 4.4 ×10 ⁻² ppb)	Speulderbos Forest, Netherlands, summer 1992	Peters et al., 1994
0–0.5 ppb	Järlsa Sweden, June 1989	Janson & Kristensson, 1991	
Air, urban/suburban	nd ^b –0.36 : g/m ³ (nd–6.4×10 ⁻² ppb)	Urban Riverside, California, June 1990	Helmig & Arey, 1992
	0.14 ng/litre (2.5×10 ⁻² ppb)	Montelibretti, Italy (suburban site)	Ciccoioli et al., 1992
	0–5.7 ppb (0–31.7 : g/m ³)	Houston, Texas	Bertsch et al., 1974
	<1–11 : g/m ³ (<0.2–1.9 ppb)	Rural, suburban and urban sites in Northern Italy, 1983–1984 (mean 1 : g/m ³ , or 0.2 ppb)	De Bortoli et al., 1986
	detected	Leningrad, Russia, summer–autumn, 1976	Ioffe et al., 1977
	detected	Denver, Colorado, USA, Jan.–Feb. 1984	Hutte et al., 1984
	nd–2.0 ng/m ³ (nd–3.5×10 ⁻⁴ ppb)	Tübingen, Germany, March–April 1985 (suburban)	Jüttner, 1988
detected	Six larger cities ^c in USSR, 1977	Ioffe et al., 1979	
Air, emissions	1.7–10 100 : g/m ³ (0.3–1.8×10 ³ ppb)	8 municipal solid waste composting facilities, USA	Eitzer, 1995
	2–240 mg/m ³ (3.5×10 ² – 4.1×10 ⁴ ppb)	8 landfill sites, UK (mean approx. 101 mg/m ³ , or 1.8×10 ⁴ ppb)	Young & Parker, 1983, 1984
	detected	Refuse waste, Singapore	Koe & Ng, 1987
	1.9–14 : g/m ³ (0.34–2.5 ppb)	Emission plumes from kraft pulp industries, Sweden	Strömvall, 1992
3.8–39 : g/m ³ (0.67–6.9 ppb)	Ambient air downwind from stone groundwood production, Sweden, 1989	Strömvall, 1992	
Water, sea	2–40 ng/litre	Gulf of Mexico, ^d 1977	Sauer, 1981
	0.55 ng/litre (mean)	Barcelona, Mediterranean Sea, Spain, 1986	Gomez-Belinchon et al., 1991
	4.4 ng/litre (mean)	Vilanova-Sitges, Mediterranean Sea, Spain, 1986	Gomez-Belinchon et al., 1991
	nd–20 ng/litre	Terra Nova Bay, Antarctica, 1988–1989, seawater (mean 5.4 ng/litre)	Desideri et al., 1991
	nd–82 ng/litre	Terra Nova Bay, Antarctica, 1988–1989, particulate	Desideri et al., 1991
	84 ng/litre	Resurrection Bay, Alaska, June 1985	Button & Jüttner, 1989
0.47 ng/litre	Resurrection Bay, Alaska, June 1986	Button & Jüttner, 1989	
Water, river	590 ng/litre (mean)	Llobregat River, Barcelona, Spain, 1985–1986	Gomez-Belinchon et al., 1991
	1600 ng/litre (mean)	Besós River, Barcelona, Spain, 1985–1986	Gomez-Belinchon et al., 1991
	detected	Black Warrior River, Tuscaloosa, USA, 1975	Bertsch et al., 1975
	detected	River Lee, London, UK	Waggott, 1981
	detected	River Glatt, Switzerland, 1975	Zürcher & Giger, 1976
Water, estuary	25–633 ng/litre	Southampton Water estuary, UK	Bianchi et al., 1991

Table 3: Continued

Medium	Concentration	Location and sampling date	Reference
Water, groundwater	70 ng/litre (max.)	Otis Air Base, Massachusetts (sewage-contaminated water)	Barber et al., 1988
	1–130 : g/litre	Former site for production of charcoal and pine tar products, Gainesville, Florida	McCreary et al., 1983
Water, drinking-water	0.03 : g/litre	13 cities in USA (detected in 1 of 13 cities)	Keith et al., 1976
	detected	UK (detected in 5 of 14 samples)	Fielding et al., 1981
	187 : g/kg (1.87×10 ⁵ ng/litre)	Canada, bottled drinking-water (detected in 1 of 182 samples)	Page et al., 1993
Water, wastewater, and landfill leachate	nd–20 : g/litre	Influent waste water, sewage works, Göteborg, Sweden, 1989–1991	Paxéus et al., 1992
	10–220 ppb (10×10 ³ – 220×10 ³ ng/litre)	Kraft mill aerated lagoons, USA	Wilson & Hrutfiord, 1975
	nd	Effluent wastewater, sewage works, Göteborg, Sweden, 1989–1991	Paxéus et al., 1992
	detected	Industrial landfill leachate, USA	Venkataramani & Ahlert, 1984
Ice	4–15 ng/litre	Terra Nova Bay, Antarctica, 1988–1989, pack ice, (mean 8 ng/litre)	Desideri et al., 1991
Sediment	105–807 ng/kg	Southampton Water estuary, UK	Bianchi et al., 1991
Soil	nd–920 : g/g	Former site for production of charcoal and pine tar products, Gainesville, Florida, USA	McCreary et al., 1983
Litter	4.0 : g/g (mean)	Litter of single leaf pinyon woodlands, Western Great Basin, USA	Wilt et al., 1988
Fish	detected	Carp from Las Vegas Wash, USA	Hiatt, 1983
	nd	Rainbow trout from Colorado River, USA	Hiatt, 1983

^a Average concentration of terpenes, on a particulate carbon basis.

^b Not detected.

^c Baku, Kemerovo, Leningrad, Murmansk, Tashkent, and Tblisi.

^d Near the mouth of the Mississippi River and on the Louisiana Shelf.

based on the daily inhalation volume for adults of 22 m³, a mean body weight for males and females of 64 kg, the assumption that 4 of 24 hours are spent outdoors (IPCS, 1994), and arithmetic mean limonene levels in indoor and outdoor air of 0.04 and 0.002 mg/m³, respectively, in a study from Los Angeles (Wallace et al., 1991).

Data on concentrations of limonene in drinking-water are limited. However, the intake of limonene from drinking-water is likely to be negligible owing to its low solubility. Dermal exposure to limonene by the general population is mainly from contact with household cleaning products in which limonene is a fragrance additive. The dermal uptake of *d*-limonene by humans is likely to be low compared with that via inhalation (Falk et al., 1991).

Inhalation is the principal route of occupational exposure to limonene. According to the National Exposure Database in Norway, concentrations of limonene between 1985 and 1992 in the occupational environment ranged from 0 to 886 mg/m³ (mean 28 mg/m³) (Fjelstad & Wolbæk, 1992). In a study from Sweden, occupational concentrations ranged from 0.9 to 400 mg/m³ (Carlsson et al., 1991). There is also a potential for dermal exposure to limonene in the occupational environment, although quantitative data are not available.

The estimated intake of limonene from occupational exposure was calculated on the same basis as for indoor and outdoor air, assuming that 8 of 24 hours are spent in the workplace each day, with an air concentration of 150 mg/m³, which is the occupational exposure limit value in Sweden (National Board of Occupational Safety and Health, 1993). The intake of limonene associated with working at the occupational exposure limit was estimated as 17 mg/kg body weight per day.

7. COMPARATIVE KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

d-Limonene has a high partition coefficient between blood and air ($\mathcal{R}_{\text{blood/air}} = 42$) and is easily taken up in the blood at the alveolus (Falk et al., 1990). The net uptake of *d*-limonene in volunteers exposed to the chemical at concentrations of 450, 225, and 10 mg/m³ for 2 hours during light physical exercise averaged 65% (Falk Filipsson et al., 1993). Orally administered *d*-limonene is rapidly and almost completely taken up from the gastrointestinal tract in humans as well as in animals (Igimi et al., 1974; Kodama et al., 1976). Infusion of labelled *d*-limonene into the common bile duct of volunteers revealed that the chemical was very poorly

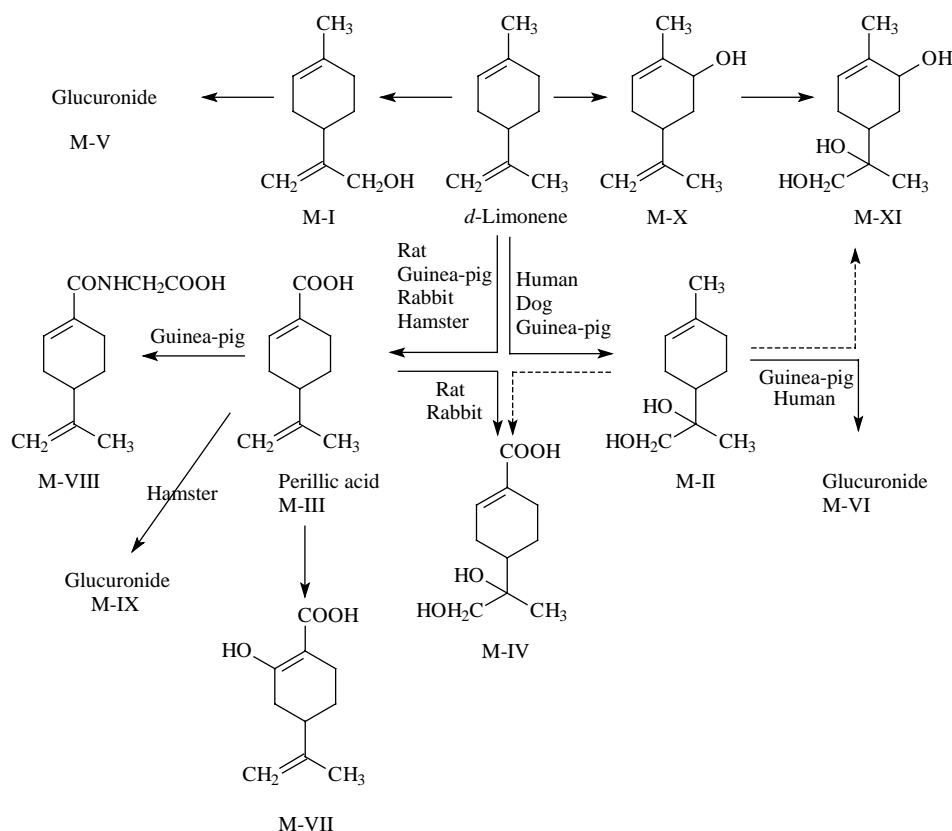
absorbed from the biliary system (Igimi et al., 1991). In shaved mice, the dermal absorption of [³H]*d*/*l*-limonene from bathing water was rapid, reaching the maximum level in 10 minutes (von Schäfer & Schäfer, 1982). In one study (one hand exposed to 98% *d*-limonene for 2 hours), the dermal uptake of *d*-limonene in humans was reported to be low compared with that by inhalation (Falk et al., 1991); however, quantitative data were not provided.

d-Limonene is rapidly distributed to different tissues in the body and is readily metabolized. Clearance from the blood was 1.1 litre/kg body weight per hour in males exposed for 2 hours to *d*-limonene at 450 mg/m³ (Falk Filipsson et al., 1993). A high oil/blood partition coefficient and a long half-life during the slow elimination phase suggest high affinity to adipose tissues (Falk et al., 1990; Falk Filipsson et al., 1993). In rats, the tissue distribution of radioactivity was initially high in the liver, kidneys, and blood after the oral administration of [¹⁴C]*d*-limonene (Igimi et al., 1974); however, negligible amounts of radioactivity were found after 48 hours. Differences between species regarding the renal disposition and protein binding of *d*-limonene have been observed. For rats, there is also a sex-related variation (Lehman-McKeeman et al., 1989; Webb et al., 1989). The concentration of *d*-limonene equivalents was about 3 times higher in male rats than in females, and about 40% was reversibly bound to the male rat specific protein, "2: -globulin (Lehman-McKeeman et al., 1989; Lehman-McKeeman & Caudill, 1992).

The biotransformation of *d*-limonene has been studied in many species, with several possible pathways of metabolism (Figure 1). Metabolic differences between species have been observed with respect to the metabolites present in both plasma and urine. About 25–30% of an oral dose of *d*-limonene in humans was found in urine as *d*-limonene-8,9-diol and its glucuronide; about 7–11% was eliminated as perillic acid (4-(1-methylethenyl)-1-cyclohexene-1-carboxylic acid) and its metabolites (Smith et al., 1969; Kodama et al., 1976). *d*-Limonene-8,9-diol is probably formed via *d*-limonene-8,9-epoxide (Kodama et al., 1976; Watabe et al., 1981). In another study, perillic acid was reported to be the principal metabolite in plasma in both rats and humans (Crowell et al., 1992). Other reported pathways of limonene metabolism involve ring hydroxylation and oxidation of the methyl group (Kodama et al., 1976).

Following the inhalation exposure of volunteers to *d*-limonene at 450 mg/m³ for 2 hours, three phases of elimination were observed in the blood, with half-lives of about 3, 33, and 750 minutes, respectively (Falk Filipsson et al., 1993). About 1% of the amount taken up was eliminated unchanged in exhaled air, whereas about 0.003% was eliminated unchanged in the urine. When male volunteers were administered (per os) 1.6 g

Figure 1. Possible pathways of *d*-limonene



From Kodayama *et al* (1976). M-I, *p*-Mentha-1,8-dien-10-ol; M-II, *p*-menth-1-ene-8,9-diol; M-IV, perillic acid-8,9-diol; M-V, *p*-mentha-1,8-dien-10-yl- β -D-glucopyranosiduronic acid; M-VI, 8-hydroxy-*p*-menth-1-en-9-yl- β -D-glucopyranosiduronic acid; M-VII, 2-hydroxy-*p*-menth-8-en-7-oic acid; M-VIII, perillylglycine, M-IX, perillyl- β -D-glucopyranosiduronic acid; M-X, *p*-mentha-1,8-dien-6-ol; M-XI, *p*-menth-1-ene-6,8,9-triol.

[¹⁴C]*d*-limonene, 50–80% of the radioactivity was eliminated in the urine within 2 days (Kodama *et al.*, 1976). Limonene has been detected, but not quantified, in breast milk of non-occupationally exposed mothers (Pellizzari *et al.*, 1982).

8. EFFECTS ON LABORATORY MAMMALS AND *IN VITRO* TEST SYSTEMS

8.1 Single exposure

The acute toxicity of *d*-limonene in rodents is fairly low after oral, intraperitoneal, subcutaneous, and intravenous administration, based on the magnitude of the LD₅₀ values (Table 4). LD₅₀ values were approximately 5 g/kg body weight for the oral administration of *d*-limonene or *d/l*-limonene to rats and for dermal application of *d/l*-limonene to rabbits and 6 g/kg body weight for oral administration to mice (Tsuji *et al.*, 1974, 1975b; Opdyke, 1978). Studies on the acute inhalation toxicity of limonene were not identified.

Effects observed following the acute exposure of rodents to limonene include increased bile flow at 85 mg/kg body weight (Kodama *et al.*, 1976), inhibition of *S*-3-hydroxy-3-methylglutaryl-CoA reductase activity at 409 mg/kg body weight (Clegg *et al.*, 1980), enzyme induction at 600 and 1200 mg/kg body weight (Ariyoshi *et al.*, 1975), and decreased motor activity, hypothermia, and potentiation of hexobarbital-induced sleep at 3 ml/kg body weight (Tsuji *et al.*, 1974).

8.2 Irritation and sensitization

d-Limonene is considered a skin irritant (Cronin, 1980; Fischer, 1986). The skin irritancy of limonene in guinea-pigs (Klecak *et al.*, 1977) and rabbits (Lacy *et al.*, 1987; Okabe *et al.*, 1990) is considered moderate and low, respectively. In an *in vivo* study of rabbit skin irritation, *d*-limonene was ranked 3.5 of 8 on the basis of the primary irritation index (Bagley *et al.*, 1996); effects were graded according to OECD Test Guideline 404 (OECD, 1993). In a study in rabbits, *d*-limonene caused irritation to the eyes (Tsuji *et al.*, 1974).

Although *d*-limonene was once considered the main allergen in citrus fruits, data from more recent

Table 4: Acute toxicity of limonene.

Species (sex)	Route of administration	Type of limonene	LD ₅₀ (g/kg body weight)	Reference
rabbit	dermal	d/l	>5	Opdyke, 1978
rat	oral	d/l	5.3	Opdyke, 1978
rat (m/f)	oral	d	4.4/5.1	Tsuji et al., 1975b
rat (m/f)	intraperitoneal	d	3.6/4.5	Tsuji et al., 1975b
rat (m/f)	intravenous	d	0.125/0.11	Tsuji et al., 1975b
mouse (m/f)	oral	d	5.6/6.6	Tsuji et al., 1975b
mouse (m/f)	oral, 7 days	d	5.3/6.8 ^a	Tsuji et al., 1974
mouse (m/f)	intraperitoneal, 3 days	d	3.1/3.0 ^a	Tsuji et al., 1974
mouse (m + f)	intraperitoneal	d	1.3	Tsuji et al., 1975b
mouse (m/f)	intraperitoneal, 10 days	d	0.59/0.50 ^a	Tsuji et al., 1974
mouse (m + f)	subcutaneous	d	>41.5	Tsuji et al., 1975b
mouse (m + f)	subcutaneous, 7 days	d	>21.5	Tsuji et al., 1974

^a Calculated from ml/kg body weight.

studies in animals have revealed air-oxidized *d*-limonene, rather than unoxidized *d*-limonene, to be the sensitizing agent. When limonene (unspecified form and unknown purity of the test material) was tested in four different sensitization assays with guinea-pigs (Open Epicutaneous Test, Maximization Test, Draize's Test, and a test with Freund's Complete Adjuvant), it was sensitizing in all but Draize's Test (Klecak et al., 1977). In another study in mice, *d*-limonene did not induce sensitization (Maisey & Miller, 1986). Hydroperoxides and other oxidation products of *d*-limonene formed on exposure to the air have proved to be potent contact allergens when tested with Freund's Complete Adjuvant in guinea-pigs, whereas unoxidized *d*-limonene did not cause any sensitization (Karlberg et al., 1991, 1992).

8.3 Short-term exposure

Increases in hepatic cytochrome P-450 content have been observed in female rats administered limonene (isomer unspecified; 40 mg/kg body weight per day for 3 days) by intraperitoneal injection (Austin et al., 1988) and in rats administered 5% *d*-limonene in the diet for 2 weeks (Maltzman et al., 1991). Increased epoxide hydratase activity was observed in rats administered 1% or 5% *d*-limonene in the diet for 2 weeks (Maltzman et al., 1991). Increases in phase II enzymes (glutathionyltransferase and UDP-glucuronyltransferase) during the exposure of rats to 5% limonene in food have also been described (Maltzman, 1991). Increased relative liver weight (from 5 to 20 times) has been observed in rats administered *d*-limonene at a dose of 75–300 mg/kg body weight; at 300 mg/kg body weight, the increase was significant (Kanerva et al., 1987b). In cats, infusion of 97% *d*-limonene into the bile system to dissolve gallstones caused acute and chronic inflammatory changes (Schenk et al., 1980).

8.4 Long-term exposure

8.4.1 Subchronic exposure

Peroral administration of *d*-limonene to rats at a dose of 400 mg/kg body weight for 30 days resulted in a 20–30% increase in the amount and activity of different liver enzymes (cytochrome P-450, cytochrome b5, aminopyrine demethylase, and aniline hydroxylase), increased relative liver weight, and decreased cholesterol levels (Ariyoshi et al., 1975). Administration of *d*-limonene (0, 2, 5, 10, 30, and 75 mg/kg body weight per day) by gavage to groups of 10 male rats, 5 days/week for 13 weeks (Webb et al., 1989), resulted in the pathological formation of granular casts at the outer zone of the renal medulla. The no-observed-effect level (NOEL), based upon histological examination of the kidneys, was considered to be 5 mg/kg body weight per day. The LOEL for increased liver and kidney weight was 75 mg/kg body weight per day, the highest dose tested. The NOEL for effects in the liver was 10 mg/kg body weight; the no-observed-adverse-effect level (NOAEL) for effects in the liver was 30 mg/kg body weight per day. Linear regression analysis revealed a dose-related trend in the increased relative weights of the kidney and liver at 30 and 75 mg/kg body weight per day. No histopathological changes were observed in the liver in these two studies. The amount and activity of different liver enzymes were not investigated, and thus the increase in relative liver weight may be due to enzyme induction.

8.4.2 Chronic exposure and carcinogenicity

The oral administration of *d*-limonene (0.4, 1.2, or 3.6 ml/kg body weight per day) to dogs for 6 months caused nausea and vomiting (Tsuji et al., 1975a). A 35% increase in alkaline phosphatase and cholesterol in serum and slightly increased total and relative liver weights were observed in dogs after peroral administra-

tion of *d*-limonene at a dose of 1.2 ml/kg body weight per day for 6 months (about 1000 mg/kg body weight per day) (Webb et al., 1990).

In a 2-year study, *d*-limonene was administered (per os) 5 days/week to groups of 50 F344/N rats (0, 75, or 150 mg/kg body weight per day to males, and 0, 300, or 600 mg/kg body weight per day to females) and B6C3F₁ mice (0, 250, or 500 mg/kg body weight per day to males, and 0, 500, or 1000 mg/kg body weight per day to females) (NTP, 1990). Slightly lower body weights were observed for rats in the high-dose groups and female mice in the high-dose group; however, no clinical symptoms could be related to the administration of *d*-limonene. For female rats in the high-dose group, survival was reduced after 39 weeks (NTP, 1990). There was clear evidence of carcinogenic activity of *d*-limonene in male rats, based upon a dose-related increase in the incidence of hyperplasia and adenoma/adenocarcinoma in renal tubular cells. However, there was no evidence of carcinogenicity in female rats or in male and female mice. The carcinogenic response in the kidney of male rats has been linked to a unique renal perturbation involving "2: -globulin.

To determine whether *d*-limonene would cause a sustained increase in renal cell proliferation and exhibit promoting activity for the development of renal adenomas in male F344 rats, the animals were administered (by stomach tube) *d*-limonene (150 mg/kg body weight per day) as a promoter 5 days/week for 30 weeks (Dietrich & Swenberg, 1991). *N*-ethyl-*N*-hydroxyethylnitrosamine (500 ppm) was used as an initiator in the drinking-water for 2 weeks. In addition, male "2: -globulin-deficient rats were exposed in the same manner to determine if the male rat specific urinary protein "2: -globulin is required for *d*-limonene to cause these effects. Exposure to *d*-limonene alone caused a significant increase in the number of atypical tubules and atypical hyperplasias in F344 rats, compared with vehicle controls. There was no increase in the incidence of tumours or preneoplastic lesions in the "2: -globulin-deficient rats exposed to *d*-limonene, whereas a 10-fold increase in the incidence of renal adenoma and atypical hyperplasia was observed in F344 rats exposed to *d*-limonene, compared with controls. There was a significant decrease in the incidence of liver tumours in animals exposed to *N*-ethyl-*N*-hydroxyethylnitrosamine and *d*-limonene, compared with *N*-ethyl-*N*-hydroxyethylnitrosamine exposure alone.

8.5 Genotoxicity and related end-points

On the basis of available data, there is no evidence that *d*-limonene or its metabolites are genotoxic or mutagenic. Limonene and its epoxides were not mutagenic when tested at concentrations of 0.3–3333 : g/plate in *in vitro* assays using different strains of *Salmonella typhimurium*, in the presence or absence of metabolic

activation (Florin et al., 1980; Watabe et al., 1981; Haworth et al., 1983; Connor et al., 1985; NTP, 1990). *d*-Limonene did not increase the frequency of forward mutation at the TK+! locus in mouse L5178Y cells (NTP, 1990), induce cytogenetic damage in Chinese hamster ovary cells (Anderson et al., 1990), or malignantly transform Syrian hamster embryo cells (Pienta, 1980). In one *in vitro* study, following exposure with benzo(*a*)pyrene, *d*-limonene (21.9 : mol/litre) inhibited the formation of transformed cell colonies in tracheal epithelium isolated from rats (Steele et al., 1990).

No evidence of mutagenicity was reported in an *in vivo* spot test with mice, involving the intraperitoneal injection of limonene at 215 mg/kg body weight per day on days 9–11 during gestation (Fahrig, 1984).

8.6 Reproductive and developmental toxicity

Studies on the reproductive toxicity of limonene were not identified. There is no evidence that limonene has teratogenic or embryotoxic effects in the absence of maternal toxicity. In rats, the oral administration of *d*-limonene (2869 mg/kg body weight per day) on days 9–15 of gestation resulted in decreased body weight and deaths among the dams. Delayed ossification and decreased total body and organ weights (thymus, spleen, and ovary) were observed in the offspring (Tsuji et al., 1975b). In mice, the oral administration of *d*-limonene (2869 mg/kg body weight per day) on days 7–12 of gestation resulted in reduced growth in the mothers and a significantly increased incidence of skeletal anomalies and delayed ossification in the offspring (Kodama et al., 1977a). The oral administration of *d*-limonene (250, 500, or 1000 mg/kg body weight per day) to rabbits on days 6–18 of gestation had no dose-related effects on the offspring. At the highest dose, there were some deaths and reduced weight gain among the dams; at the intermediate dose, growth was decreased (Kodama et al., 1977b).

8.7 Immunological and neurological effects

Reports relating limonene to type I allergy were not identified. In a study designed to assess the immunological effects of *d*-limonene on B- and T-cell responses, BALB/c mice were administered (by forced intragastric feeding) *d*-limonene (0.1 ml) daily for 9 weeks (Evans et al., 1987). Mice given keyhole limpet haemocyanin prior to exposure to *d*-limonene had suppressed primary and secondary anti-keyhole limpet haemocyanin responses. Mice exposed to *d*-limonene prior to the administration of keyhole limpet haemocyanin had significantly increased antibody and mitogen-induced proliferative responses. However, the purity of the *d*-limonene in this study was not checked,

and oxidation products may have been the active substances.

Effects on the central nervous system following exposure to limonene have been reported in experimental studies with animals; however, it is difficult to ascertain whether these effects were the result of general intoxication or a more direct effect of the chemical. The peroral administration of *d*-limonene (3 ml) to rats and mice resulted in decreased motor activity (Tsuji et al., 1974). A similar effect was also observed in mice orally administered a limonene dose of 1000 mg/kg body weight per day for 13 weeks (NTP, 1990).

9. EFFECTS ON HUMANS

Case reports or epidemiological studies on the effects of limonene on human health were not identified. Available data have been derived from studies with volunteers. In older investigations, multiple exposures and confounding factors such as mechanical damage, irritation, other allergens, and infections due to wet work (Beerman et al., 1938; Schwartz, 1938; Birmingham et al., 1951) may have contributed to the effects reported following exposure to limonene. None of eight subjects reported any discomfort, irritation, or symptoms related to central nervous system effects during a 2-hour inhalation exposure to *d*-limonene at 10, 225, or 450 mg/m³; however, a slight decline in vital capacity was observed following exposure to the highest concentration (Falk Filipsson et al., 1993).

In a study in which the sensitivity of four patch testing systems (Finn chamber, Hill Top patch, Van der Bend chamber, and Webril patch) was evaluated in volunteers, *d*-limonene (perfume-grade) reacted strongly in all types of patches within 10–15 minutes of exposure (York et al., 1995). Skin irritation was assessed before application, as well as immediately and 1, 24, 48, and 72 hours after removal of the patch, using a scoring system based broadly on that used for rabbit irritation studies (OECD, 1993), but modified to account for the nature of reactions on human skin. There was evidence of sensory effects and urticarial responses on removal of the patches. Significant irritation persisted for 24 hours, and these reactions persisted for 48 and 72 hours in many volunteers (York et al., 1995). Dermal exposure to *d*-limonene (98%) for 2 hours in one subject caused burning, itching, aching, and a long-lasting purpuric rash (Falk et al., 1991).

d-Limonene infused directly into the bile system of human volunteers to dissolve gallstones caused pain in the upper abdomen, nausea, vomiting, and diarrhoea, as well as increases in serum aminotransferases and alkaline

phosphatase (Igimi et al., 1976, 1991). The oral administration of 20 g *d*-limonene to volunteers resulted in diarrhoea, painful constrictions, and proteinuria, but no biochemical changes (total protein, bilirubin, cholesterol, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase) in the liver (Igimi et al., 1976). Reports of contact allergy to dipentene have appeared (Calnan, 1979; Rycroft, 1980). In one investigation, 15 of 22 people with an allergy to oil of turpentine also reacted to dipentene (Cachao et al., 1986). Patch testing in consecutive dermatitis patients from Sweden and Belgium revealed positive reactions in 1.5–2% of the subjects tested with oxidized *d*-limonene, a finding similar to that observed with other common sensitizers, such as formaldehyde (A.-T. Karlberg, personal communication, 1996). *d*-Limonene reduced non-immunological contact urticaria caused by cinnamic aldehyde, with competitive receptor inhibition suggested as the mechanism of suppression (Guin et al., 1984). No sensitizing effect was observed when 25 volunteers were exposed to *d*-limonene in a Human Maximization Test (Grief, 1967).

10. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

10.1 Aquatic environment

The acute toxicity of *d*-limonene ranges from slight to high for aquatic organisms (Table 5). The lowest acute toxicity values (EC₅₀ or LC₅₀) identified were approximately 0.4 mg/litre for *Daphnia* (US EPA, 1990b) and 0.7 mg/litre for fish (US EPA, 1990a,b). The no-observed-effect concentration (NOEC) for green algae is approximately 4 mg/litre (US EPA, 1990a). The acute toxicity (EC₅₀ or LC₅₀) of dipentene to *Daphnia* and fish is about 50–70 times lower than that for *d*-limonene (US EPA, 1990b). No studies were identified on the chronic toxicity of limonene to aquatic organisms.

10.2 Terrestrial environment

The toxicity of limonene has been studied in various terrestrial organisms (Table 6). Limonene generally has moderate acute toxicity in insects and mites. The acute toxicity of *d*-limonene to earthworms (*Eisenia foetida* Savigny) was high (LC₅₀ = 6.0 ppm; mg/kg) (Karr et al., 1990). Sublethal effects (i.e. abnormal rebounding of medial giant fibre pathway [MGF] impulses and spontaneous lateral giant fibre pathway [LGF] spiking) were observed following exposure of earthworms to 4.2 ppm (mg/kg) limonene (Karr et al., 1990). Limonene has low subacute toxicity to bobwhite quail (*Colinus virginianus*) exposed via the diet (LC₅₀ > 5620 ppm; mg/kg) (US EPA, 1994).

Table 5: Toxicity of limonene to aquatic organisms.

Species	End-point; exposure	Results (mg/litre)	Reference
Algae			
Green algae ^a	96-h NOEC; static	4.08	US EPA, 1990a
Crustaceans			
Water flea (<i>Daphnia magna</i>) ^b	48-h LC ₅₀ ; flow-through 48-h EC ₅₀ ; flow-through	0.577 (0.496–0.672) 0.421	US EPA, 1990b
Water flea (<i>D. magna</i>) ^c	acute LC ₅₀	39 ppm	US EPA, 1994
Water flea (<i>D. magna</i>) ^a	48-h LC ₅₀ ; flow-through 48-h EC ₅₀ ; flow-through	31 (27.5–34.8) 28.2	US EPA, 1990b
Water flea (<i>Daphnia pulex</i>) ^b	48-h EC ₅₀ ; flow-through	0.730	US EPA, 1990a
Water flea (<i>D. pulex</i>) ^c	48-h EC ₅₀ ; static	69.6	Passino & Smith, 1987
<i>Daphnia</i> ^b	21-d NOEC; structure–activity relationship (SAR) analysis	0.15	US EPA, 1990a
Fish			
Fathead minnow (<i>Pimephales promelas</i>) ^b	96-h LC ₅₀ ; flow-through	0.702 (0.619–0.796)	US EPA, 1990b
Fathead minnow (<i>P. promelas</i>) ^b	96-h LC ₅₀ ; flow-through 96-h EC ₅₀ ; flow-through	0.720 (0.618–0.839) 0.688 (0.606–0.782)	US EPA, 1990b
Fathead minnow (<i>P. promelas</i>) ^a	96-h LC ₅₀ ; flow-through 96-h EC ₅₀ ; flow-through	38.5 (35.4–41.8) 28.2	US EPA, 1990a,b
Fish ^c	acute LC ₅₀	80 ppm	US EPA, 1994
Fish ^b	96-h LC ₅₀ ; flow-through	0.711	US EPA, 1990a
Golden orfe (<i>Leuciscus idus</i>) ^a	48-h LC ₅₀	32	Roth, 1990
Insects			
Water hyacinth weevil (<i>Neochetina eichhorniae</i> , 60%, <i>N. bruchi</i> , 40%) ^b	Mortality (73%, range 40–100%), weevils were dipped in limonene	50% limonene	Haag, 1986
Mosquito fly (<i>Culex quinquefasciatus</i>) ^c	2nd-instar larvae (23–33°C), 72-h LC ₅₀ ; static 4th-instar larvae (23–33°C), 72-h LC ₅₀ ; static	6.6–26.1 ppm 7.8–30.6 ppm	Mohsen et al., 1989 Mohsen et al., 1989

^a *d/l*-Limonene; ^b *d*-Limonene; ^c Optical isomer not specified.

11. EFFECTS EVALUATION

11.1 Evaluation of health effects

11.1.1 Hazard identification and dose–response assessment

Limonene is a skin irritant in experimental animals and humans. *d*-Limonene is an eye irritant in rabbits. Studies in guinea-pigs have revealed that air-oxidized *d*-limonene, but not *d*-limonene itself, induced contact allergy. Similar results are likely with *l*-limonene and dipentene.

The critical organ in animals (except for male rats) following peroral or intraperitoneal administration is the liver. Exposure to limonene affects the amount and activity of different liver enzymes, liver weight, cholesterol levels, and bile flow, with effects having been observed in mice, rats, and dogs. In male rats, exposure to *d*-limonene results in damage to the kidneys and an increased incidence of renal tumours. As the male rat specific protein "2: -globulin is considered to play a crucial role in the development of the neoplastic

and non-neoplastic kidney lesions, they are considered not relevant for human risk assessment.

A dose-related nephropathy was observed in the kidneys of male rats after oral administration of *d*-limonene (NTP, 1990). This lesion, consisting of degeneration of epithelial cells in the convoluted tubules, granular casts in the outer stripe of the outer medulla, and epithelial regeneration, is characteristic of hyaline droplet nephropathy associated with the accumulation of "2: -globulin in the cytoplasm of tubular cells (Alden et al., 1984; Halder et al., 1985) in response to a variety of hydrocarbon compounds (Swenberg et al., 1992). Some compounds fit deeply into a hydrophobic pocket of "2: -globulin. When hydrogen bonding between the chemical and protein occurs, the digestibility of "2: -globulin by proteases is inhibited, leading to accumulation of the male rat specific protein in lysosomes of the P2 segment of the nephron (Lehman-McKeeman et al., 1990). Although such chemicals fall into rather diverse classes, molecular modelling studies have demonstrated a strong structure–activity relationship with respect to "2: -globulin binding (Borghoff et al., 1991). The accumulation of "2: -globulin is cytotoxic, resulting in single-cell

Table 6: Toxicity of limonene to terrestrial organisms.

Species	End-point; exposure	Results	Reference
Insects			
Cat flea (<i>Ctenocephalides felis</i>) ^{a,b}	Adult LD ₅₀ ; contact	160 (157–163) : g/cm ²	Hink & Fee, 1986
	Adult LD ₅₀ ; vapour	259 (234–281) : g/cm ²	
	Pupae LD ₅₀ ; contact	376 (259–468) : g/cm ²	
	Larvae LD ₅₀ ; contact	226 (221–231) : g/cm ²	
	Eggs; lethal to all eggs; contact	65 : g/cm ²	
Variegated cutworm (<i>Peridroma saucia</i>) ^b	Larvae; significant inhibition of pupation; dietary exposure	0.2% limonene in artificial feed	Harwood et al., 1990
German cockroach (<i>Blattella germanica</i> L.) ^b	Adult 24-h LD ₅₀ ; topical	700 (610–810) : g/insect	Karr & Coats, 1988
	Adult 24-h LC ₅₀ ; fumigation	23.3 (17.5–31.0) ppm	
	Adult; no mortality; oral	25% limonene in feed	
	Nymph; no mortality; oral	25% limonene in feed	
German cockroach (<i>B. germanica</i> L.) ^b	Adult; no mortality; 72-h contact with treated surface	limonene (conc. not given)	Karr & Coats, 1992
	Effect on growth rate; diet	1–25% limonene in diet	
	EC ₅₀ , oothecae yielding young; topical exposure	0.68 mg/ootheca	
	No effect on reproduction; via diet	0.84 mg/cockroach	
Rice weevil (<i>Sitophilus oryzae</i> L.) ^b	Topical exposure	5 mg/litre in air	Karr & Coats, 1988
	Vapour exposure		
Rice weevil (<i>Sitophilus oryzae</i> L.) ^b	Adult 24-h LC ₅₀ ; fumigation	19.0 (13.2–27.3) ppm	Karr & Coats, 1988
House fly (<i>Musca domestica</i> L.) ^b	25-h LD ₅₀ ; topical	90 (70–130) : g/insect	Karr & Coats, 1988
Western corn rootworm (<i>Diabrotica virgifera virgifera</i> LeConte) ^b	Egg 72-h LC ₅₀ ; contact with treated substrate	1.8 (0.8–2.9)% limonene	Karr & Coats, 1988
	Larvae 72-h LC ₅₀ ; contact with treated soil	12.2 (4.5–32.6) ppm	
Spiders and allies			
Spruce spider mite (<i>Oligonychus ununguis</i> (Jacobi)), ^c adult female	24-h LC ₅₀ ; vapour	24.5 ppm	Cook, 1992
	Significant decrease in oviposition	5 ppm	
Segmented worms			
Earthworm (<i>Eisenia foetida</i> Savigny) ^b	48-h LC ₅₀	6.0 (5.1–7.1) ppm	Karr et al., 1990
	Sublethal effects	4.2 ppm	
Birds			
Bobwhite quail (<i>Colinus virginianus</i>) ^d	Subacute LC ₅₀ ; dietary exposure	>5 620 ppm	US EPA, 1994

^a Fleas were exposed to filter papers treated with limonene, either directly or to vapours from the filter papers.

^b *d*-Limonene.

^c *l*-Limonene.

^d Optical isomers not specified.

necrosis (Dietrich & Swenberg, 1991). The exfoliated renal epithelium is restored by compensatory cell proliferation. The increase in cell proliferation associated with "2: -globulin is reversible. Damage of this type has not been observed in female rats, male rats that do not produce "2: -globulin, or other mammals, such as mice, hamsters, guinea-pigs, dogs, and monkeys (Alden, 1986; Kanerva & Alden, 1987a; Swenberg et al., 1989; Webb et al., 1989, 1990; NTP, 1990; Ridder et al., 1990; Dietrich & Swenberg, 1991). The processes leading to nephropathy and the development of renal cancer by such compounds are among the best understood for non-genotoxic chemicals and strongly indicate that it is a male rat specific process. Acute and chronic renal effects induced in male rats by limonene will be unlikely to occur in any species not producing "2: -globulin or a

very closely related protein in the large quantities typically seen in the male rat (US EPA, 1991; Swenberg, 1993).

d-Limonene has been studied in a variety of short-term *in vitro* tests and has been found to be non-genotoxic. There is no evidence that limonene has teratogenic or embryotoxic effects in the absence of maternal toxicity.

11.1.2 Criteria for setting guidance values for limonene

In numerous experimental studies, exposure to limonene has been shown to affect the liver. Owing to a lack of data on *d*-limonene exposure in humans, this

organ cannot with certainty be stated as the critical organ in humans. Based on available data, food is believed to be the principal source of exposure (96%) to limonene; the contribution from ambient air is approximately 4%. The dermal uptake of limonene has not been estimated.

To calculate a tolerable intake for humans, the animal study was chosen in which effects on the liver were observed at the lowest exposure level (Webb et al., 1989). In this study, gavage administration of *d*-limonene (5 days/week for 13 weeks) to rats caused increased relative liver weight at 30 and 75 mg/kg body weight per day. The NOEL for the liver was considered to be 10 mg/kg body weight per day. Using uncertainty factors of 10 for intraspecies differences and 10 for interspecies differences, a tolerable intake for ingestion of *d*-limonene by humans of 0.1 mg/kg body weight per day may be calculated from the NOEL. A guidance value for inhalation exposure to *d*-limonene was not developed, as inhalation is an insignificant route of exposure compared with ingestion.

11.1.3 Sample risk characterization

Exposure estimates vary as a function of use patterns, and the risk characterization presented here is provided only as an example, primarily for illustrative purposes. In general, *d*-limonene could be considered (with the exception of its irritative and sensitizing properties) to be a chemical with fairly low toxicity. The calculated tolerable intake of 0.1 mg/kg body weight per day is of a similar magnitude as the estimated daily US consumption of *d*-limonene of 0.27 mg/kg body weight per day (Flavor and Extract Manufacturers Association, 1991).

11.2 Evaluation of environmental effects

Limonene and other terpenes are released in large amounts mainly to the atmosphere. When released to soil or water, limonene is expected to evaporate to air to a significant extent, owing to its high volatility. Thus, the atmosphere is the predominant environmental sink of limonene, where it is expected to rapidly undergo gas-phase reactions with photochemically produced hydroxyl radicals, ozone, and nitrate radicals. The oxidation of terpenes, such as limonene, contributes to aerosol and photochemical smog formation. Ozonolysis of limonene may also lead to the formation of hydrogen peroxide and organic peroxides, which have various toxic effects on plant cells and may be part of the damage to forests observed in the last decades (Peters et al., 1994). Emissions of biogenic hydrocarbons such as limonene and other terpenes to the atmosphere may either decrease ozone concentrations when nitrogen oxide concentrations are low or, if emissions take place in

polluted air (i.e. containing high nitrogen oxide levels), lead to an increase in ozone concentrations.

Terrestrial organisms are most likely to be exposed to limonene via the air. The few studies on terrestrial species (i.e. insects) using vapour exposure reveal effects of limonene at parts per million levels. Measured environmental concentrations are typically around 0.1–2 ppb (0.6–11 : g/m³), indicating a low risk for acute toxic effects on terrestrial organisms from direct exposure to limonene in air. At polluted sites, limonene concentrations in soil (up to 920 mg/kg soil) may exceed effect levels of soil-living organisms (e.g. earthworm, acute LC₅₀ = 6.0 ppm; mg/kg).

In the aquatic environment, limonene exhibits high acute toxicity to fish and *Daphnia*. It may also bioaccumulate. The lowest acute toxicity value identified was 0.4 mg/litre (48-hour EC₅₀ for *Daphnia*). Because concentrations of limonene in surface waters of “polluted” and “unpolluted” areas are at least about 250 and 20 000 times lower than this acute toxicity value, respectively, it is likely that limonene poses a low risk for acute toxic effects on aquatic organisms. No studies were identified on chronic effects, and therefore risks associated with chronic exposures of aquatic organisms to limonene in “polluted” waters cannot be determined.

12. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES

The International Agency for Research on Cancer (IARC, 1993) has classified *d*-limonene in Group 3 (not classifiable as to its carcinogenicity to humans) based on a lack of available data on carcinogenicity to humans and limited evidence for carcinogenicity in experimental animals.

The 41st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1993b) withdrew the existing acceptable daily intake for *d*-limonene of 0–1.5 mg/kg body weight per day (JECFA, 1993a) and in its place allocated “not specified.” On the basis of the available data, the total daily intake of the chemical arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food did not, in the opinion of the Committee, represent a health hazard. For that reason, and for the reasons stated in the individual evaluations, the establishment of an acceptable daily intake expressed in numerical form was not deemed necessary.

Information on international hazard classification and labelling is included in the International Chemical Safety Card reproduced in this document.

13. HUMAN HEALTH PROTECTION AND EMERGENCY ACTION

Human health hazards, together with preventative and protective measures and first aid recommendations, are presented in the International Chemical Safety Card (ICSC 0918) reproduced in this document.

13.1 Human health hazards

Limonene is flammable but essentially non-toxic. Repeated or prolonged contact with the oxidized chemical causes skin sensitization.

13.2 Advice to physicians

In case of poisoning, the treatment is supportive. Like other volatile oils, if the patient lives for 48 hours, complete recovery is likely; laboratory evidence of renal damage may persist for several months (Dreisbach & Robertson, 1987).

13.3 Storage

Limonene is flammable, with a flash point of 45°C. Keep the container in a cool, dry, well ventilated area, out of direct sunlight. Keep the container tightly closed to prevent oxidation of the chemical.

13.4 Spillage

In the case of a large spill, emergency personnel need to use non-sparking tools to avoid fire and explosion hazards.

14. CURRENT REGULATIONS, GUIDELINES, AND STANDARDS

Information on national regulations, guidelines, and standards is available from the International Register of Potentially Toxic Chemicals (IRPTC) legal file.

The reader should be aware that regulatory decisions about chemicals taken in a certain country can be fully understood only in the framework of the legislation of that country. The regulations and guidelines of all countries are subject to change and should always be verified with appropriate regulatory authorities before application.

D-LIMONENE**0918**
April 1997CAS No: 5989-27-5
RTECS No: GW6360000
UN No:
EC No:Carvene
(R)-4-Isopropenyl-1-methylcyclohexene
(+)-Limonene
C₁₀H₁₆
Molecular mass: 136.23

TYPES OF HAZARD/ EXPOSURE	ACUTE HAZARDS/SYMPTOMS	PREVENTION	FIRST AID/FIRE FIGHTING
FIRE	Flammable.	NO open flames, NO sparks, and NO smoking.	Powder, AFFF, foam, carbon dioxide.
EXPLOSION	Above 48°C explosive vapour/air mixtures may be formed.	Above 48°C use a closed system, ventilation, and explosion-proof electrical equipment.	In case of fire: keep drums, etc., cool by spraying with water.

EXPOSURE		STRICT HYGIENE!	
Inhalation		Ventilation.	Fresh air, rest.
Skin	Redness.	Protective gloves. Protective clothing.	Remove contaminated clothes. Rinse and then wash skin with water and soap.
Eyes	Redness.	Safety spectacles.	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then take to a doctor.
Ingestion		Do not eat, drink, or smoke during work.	Rinse mouth.

SPILLAGE DISPOSAL	PACKAGING & LABELLING
Collect leaking and spilled liquid in sealable containers as far as possible. Absorb remaining liquid in sand or inert absorbent and remove to safe place.	Symbol R: S:

EMERGENCY RESPONSE	STORAGE
Transport Emergency Card: TEC (R)-75	Fireproof. Cool. Well closed.

IMPORTANT DATA

Physical State; Appearance

COLOURLESS LIQUID, WITH CHARACTERISTIC ODOUR.

Chemical Dangers

Reacts violently with a mixture of iodine pentafluoride and tetrafluoroethylene, causing fire and explosion hazard.

Occupational Exposure Limits

TLV not established.

Routes of Exposure

The substance can be absorbed into the body by inhalation of its aerosol, through the skin and by ingestion.

Inhalation Risk

No indication can be given about the rate in which a harmful concentration in the air is reached on evaporation of this substance at 20°C.

Effects of Short-term Exposure

The substance may irritate slightly the eyes and the skin.

Effects of Long-term or Repeated Exposure

Repeated or prolonged contact may cause skin sensitization if the substance has been oxidized.

PHYSICAL PROPERTIES

Boiling point: 178°C
Melting point: -75°C
Relative density (water = 1): 0.84
Solubility in water: none

Vapour pressure, kPa at 14.4°C: 0.4
Relative vapour density (air = 1): 4.7
Flash point: 48°C
Octanol/water partition coefficient as log Pow: 4.2

ENVIRONMENTAL DATA

NOTES

ADDITIONAL INFORMATION

LEGAL NOTICE

Neither the EC nor the IPCS nor any person acting on behalf of the EC or the IPCS is responsible for the use which might be made of this information

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APPENDIX 1 — SOURCE DOCUMENT

Karlberg A-T, Lindell B (1993) Limonene. In: Beije B, Lundberg P, eds. *Criteria documents from the Nordic Expert Group 1993*. Solna, National Institute of Occupational Health, Nordic Council of Ministers, pp. 207–246 (*Arbete och Hälsa* 35).

Copies of the *Arbete och Hälsa* document on limonene (ISSN: 0346-7821; ISBN: 91-7045-240-7), prepared by the Nordic Expert Group, may be obtained from:

National Institute for Working Life
Publications Department
S-171 84 Solna
Sweden

In the peer review procedure of documents prepared in the series *Criteria documents from the Nordic Expert Group* (focused on human health effects only), one member of the Nordic Expert Group serves as primary reviewer for the first draft. A second draft is forwarded to all members of the Nordic Expert Group, who in turn consult appropriate specialists to review the document. The specialists are chosen either because they have an extended knowledge of the substance itself or because they are specialists in the critical effect area of the substance evaluated. The second review is performed by a review board, including the Nordic Expert Group participants with the *ad hoc* specialists. After revision, the document is checked again by the members of the Nordic Expert Group and the *ad hoc* experts for further comments. The review board meeting is repeated if necessary.

APPENDIX 2 — CICAD PEER REVIEW

The draft CICAD on limonene was sent for review to institutions and organizations identified by IPCS after contact with IPCS national Contact Points and Participating Institutions, as well as to identified experts. Comments were received from:

Department of Health, London, United Kingdom

Department of Public Health, Albert Szent-Gyorgyi University Medical School, Szeged, Hungary

Dirección General de Salud Ambiental, Subsecretario de Regulación y Fomento, Sanitario, San Luis Potosí, Mexico

Environmental Health Directorate, Health Canada, Ottawa, Canada

International Agency for Research on Cancer, Lyon, France

Ministry of Health, National Centre of Hygiene, Medical Ecology and Nutrition, Sofia, Bulgaria

Ministry of Health and Welfare, International Affairs Division, Government of Japan, Tokyo, Japan

National Institute for Working Life, Solna, Sweden

National Institute of Public Health, Oslo, Norway

United States Department of Health and Human Services (National Institute of Environmental Health Sciences)

United States Environmental Protection Agency (Office of Pollution Prevention and Toxics; Office of Drinking Water)

APPENDIX 3 — CICAD FINAL REVIEW BOARD

Brussels, Belgium, 18–20 November 1996

Members

Dr A. Aitio, Institute of Occupational Health, Helsinki, Finland

Dr K. Bentley, Director, Environment Policy Section, Commonwealth Department of Human Services and Health, Canberra, Australia

Mr R. Cary, Toxicology and Existing Substances Regulation Unit, Health and Safety Executive, Merseyside, United Kingdom

Dr J. de Fouw, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands

Dr C. DeRosa, Director, Division of Toxicology, Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA

Dr S. Dobson, Institute of Terrestrial Ecology, Monks Wood, Abbots Ripton, Huntingdon, Cambridgeshire, United Kingdom

Dr W. Farland, Director, National Center for Environmental Assessment, Office of Research and Development, US Environmental Protection Agency, Washington, DC, USA (*Chairperson*)

Dr T.I. Fortoul, Depto. Biología Celular y Tisular, National University of Mexico and Environmental Health Directorate of the Health Ministry, Mexico D.F., Mexico

Dr H. Gibb, National Center for Environmental Assessment, US Environmental Protection Agency, Washington, DC, USA

Dr R.F. Hertel, Federal Institute for Health Protection of Consumers & Veterinary Medicine, Berlin, Germany

Mr J.R. Hickman, Environmental Health Directorate, Health Canada, Ottawa, Ontario, Canada

Dr T. Lakhansky, Head, Division of Toxicology, Institute of Hygiene and Epidemiology, Brussels, Belgium (*Vice-Chairperson*)

Dr I. Mangelsdorf, Documentation and Assessment of Chemicals, Fraunhofer Institute for Toxicology and Aerosol Sciences, Hanover, Germany

Ms E. Meek, Head, Priority Substances Section, Environmental Health Directorate, Health Canada, Ottawa, Ontario, Canada

Dr K. Paksy, National Institute of Occupational Health, Budapest, Hungary

Mr D. Renshaw, Department of Health, London, United Kingdom

Dr J. Sekizawa, Division of Chemo-Bio Informatics, National Institute of Hygienic Sciences, Tokyo, Japan

Dr H. Sterzl-Eckert, GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, Institut für Toxikologie, Oberschleissheim, Germany

Professor S. Tarkowski, Department of Environmental Health Hazards, The Nofer Institute of Occupational Medicine, Lodz, Poland

Dr M. Wallen, National Chemicals Inspectorate (KEMI), Solna, Sweden

Observers

Professor F.M.C. Carpanini,¹ Director, Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Brussels, Belgium

Mr R. Haigh,¹ Head of Unit, Health and Safety Directorate, European Commission, Luxembourg

Mr B.U. Hildebrandt, Federal Ministry for the Environment, Nature Conservation and Nuclear Safety, Bonn, Germany

Mr P. Hurst,¹ Chemical and Consumer Policy Officer, Conservation Policy Division, World Wide Fund for Nature, Gland, Switzerland

Dr A. Lombard (Representative of CEFIC), ELF-ATOCHEM, Paris, France

Dr P. McCutcheon,¹ Environment, Consumer Protection and Nuclear Safety, European Commission, Brussels, Belgium

Dr R. Montaigne, Counsellor, Technical Affairs Department, European Chemical Industry Council (CEFIC), Brussels, Belgium

Dr M. Pemberton, ICI Acrylics, Lancashire, United Kingdom

Dr A. Smith, Organisation for Economic Co-operation and Development, Environment Division, Paris, France

Secretariat

Dr M. Baril, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

Dr L. Harrison, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

Dr M. Mercier, Director, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

Dr P. Toft, Associate Director, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

¹ Invited but unable to attend.

RÉSUMÉ D'ORIENTATION

Les informations contenues dans le présent CICAD (document international succinct sur l'évaluation des risques chimiques) sur le limonène (*d*-limonène, *l*-limonène et *d/l* limonène) proviennent principalement d'une étude effectuée en 1993 pour le Nordic Expert Group (Karlberg & Lindell, 1993). D'autres données pour l'évaluation du limonène ont été rassemblées à partir d'une deuxième étude menée sous les auspices du Conseil des Ministres des Pays nordiques (Josefsson, 1993), d'un rapport préliminaire (qui n'a pas fait l'objet d'une évaluation par les pairs) sur la présence et les effets du limonène dans l'environnement (US EPA, 1994), et de recherches effectuées dans les bases de données pertinentes pour les années 1993–1995. Une dernière recherche bibliographique portant sur les années 1996–1997 n'a pas fourni d'éléments de nature à modifier les conclusions formulées dans le CICAD. L'appendice 1 donne des informations sur la nature et la disponibilité des sources documentaires. Des informations concernant l'examen par les pairs du présent CICAD figurent à l'appendice 2. La publication de ce CICAD a été approuvée à une réunion du Comité d'Évaluation finale qui s'est tenue à Bruxelles (Belgique) du 18 au 20 novembre 1996. La liste des participants à cette réunion figure à l'appendice 3. La fiche internationale de sécurité chimique concernant le *d*-limonène (ICSC 0918), établie par le Programme international sur la sécurité chimique (IPCS, 1993), est également reproduite dans le présent document. L'accent a été mis sur le *d*-limonène en raison de l'abondance des données disponibles sur cet isomère.

Le limonène se trouve à l'état naturel dans certains arbres et arbustes. Le limonène et d'autres monoterpènes sont libérés en grandes quantités, principalement dans l'atmosphère, à partir de sources biologiques naturelles et de sources artificielles. Le limonène est utilisé comme solvant dans le dégraissage des métaux avant peinture, comme agent nettoyant dans les industries de l'électronique et de l'imprimerie et comme solvant pour peintures. Il est également employé comme aromatisant dans les aliments, les produits d'entretien ménagers et les parfums.

Le limonène est un irritant de la peau, tant pour l'homme que pour les animaux de laboratoire. Chez le lapin, le *d*-limonène est un irritant oculaire. Des études effectuées sur des cobayes ont montré que le *d*-limonène oxydé par l'air, mais non le *d*-limonène lui-même, induisait des allergies de contact. Étant donné que le *d*-limonène et le *l*-limonène sont des énantiomères, il pourrait en être de même pour le *l*-limonène et le dipentène (mélange des deux). Le caractère allergène du limonène pourrait donc dépendre dans une large mesure

des manipulations qu'il a subies, de sa pureté et de l'adjonction éventuelle d'antioxygènes.

L'organe critique chez l'animal (sauf chez le rat mâle), après administration par voie orale ou intrapéritonéale, est le foie. On n'a pas trouvé d'études au cours desquelles des animaux auraient été exposés au limonène par inhalation. L'exposition au limonène a des effets sur la quantité et l'activité de diverses enzymes hépatiques, ainsi que sur le poids du foie, la cholestérolémie et la sécrétion biliaire. Ces effets ont été observés chez la souris, le rat et le chien. Les données disponibles sont insuffisantes pour déterminer quel est l'organe critique chez l'homme.

Chez le rat mâle, l'exposition au *d*-limonène provoque des lésions et des tumeurs rénales. Il est admis qu'une protéine spécifique du rat mâle, l' α_2 -globuline, joue un rôle crucial dans le développement des lésions rénales néoplasiques ou non néoplasiques chez cet animal, de sorte que leur étude n'est pas jugée pertinente pour l'évaluation du risque chez l'homme. Le *d*-limonène a été soumis à une série d'épreuves *in vitro* à court terme dans lesquelles il s'est révélé non génotoxique. Il n'y a pas de preuve que le limonène exerce des effets tératogènes ou embryotoxiques en l'absence de toxicité maternelle. De façon générale, on peut admettre que le *d*-limonène est un composé de toxicité relativement faible (si l'on excepte ses propriétés irritantes et sensibilisantes).

D'après les données disponibles, les aliments sont la principale source d'exposition au limonène. Une valeur guide de 0,1 mg/kg de poids corporel par jour a été établie pour l'ingestion. D'après les estimations actuelles concernant le niveau d'exposition, la présence de limonène dans les aliments ne semble pas présenter un risque significatif pour la santé humaine.

Dans l'atmosphère, le limonène et d'autres terpènes réagissent rapidement avec les radicaux hydroxyle et nitrate résultant de réactions photochimiques, ainsi qu'avec l'ozone. L'oxydation des terpènes, dont le limonène, contribue à la formation d'aérosols et de smogs photochimiques. Dans le sol, la mobilité du limonène devrait théoriquement être faible; dans le milieu aquatique, il devrait se lier fortement aux sédiments. Le limonène est résistant à l'hydrolyse. Il est biodégradable en conditions d'aérobiose, mais non en milieu anaérobie.

Pour les organismes terrestres, l'air est la voie d'exposition la plus probable. Les quelques études effectuées sur des espèces terrestres (des insectes) exposées aux vapeurs de limonène ont révélé que celui-ci produisait des effets à des concentrations de l'ordre de quelques parties par million. Les concentrations mesurées dans l'environnement sont généralement de

l'ordre de 0,1–2 ppb (0,6–11 : g/m³). En cas de pollution, les concentrations dans le sol peuvent dépasser les niveaux pour lesquels on constate des effets sur les organisme vivants (par exemple, les vers de terre). En milieu aquatique, des signes de toxicité aiguë peuvent être observés sur les poissons et les daphnies. Les concentrations de limonène dans les eaux de surface sont généralement bien inférieures aux doses toxiques aiguës déterminées expérimentalement; il est donc peu probable que le limonène présente un risque de toxicité aiguë pour les organismes aquatiques. Aucune étude n'a été découverte sur ses effets chroniques.

RESUMEN DE ORIENTACIÓN

Esta reseña de la evaluación química internacional del limoneno (*d*-limoneno, *l*-limoneno y *d/l*-limoneno) se basa principalmente en un examen preparado en 1993 para el Grupo Nórdico de Expertos (Karlberg & Lindell, 1993). Un segundo examen efectuado bajo los auspicios del Consejo Nórdico de Ministros (Josefsson, 1993), una fuente de información preliminar, no revisada por expertos, sobre la exposición y los efectos ambientales (EPA de los Estados Unidos, 1994) y múltiples consultas en las bases de datos pertinentes sobre los años 1993 a 1995 permitieron identificar datos adicionales para evaluar el limoneno. En una última consulta de las publicaciones aparecidas en 1996 y 1997 no se hallaron datos que modificaran las conclusiones enunciadas en la reseña de la evaluación química internacional. En el apéndice 1 figura información sobre la naturaleza y disponibilidad del documento de base. En el apéndice 2 se da información sobre el examen colegiado de la presente reseña. Esta reseña fue aprobada para su publicación en una reunión del Comité de Revisión Final celebrada en Bruselas (Bélgica) del 18 al 20 de noviembre de 1996. Los participantes en la reunión figuran en el apéndice 3. Se ha reproducido asimismo la ficha internacional de seguridad química (ICSC 0918) del *d*-limoneno, elaborada por el Programa Internacional de Seguridad de las Sustancias Químicas (IPCS, 1993). La atención especial prestada al *d*-limoneno obedece a la gran cantidad de datos disponibles sobre esta forma isomérica.

El limoneno está presente en forma natural en algunos árboles y arbustos. Fuentes biógenas y antropógenas liberan limoneno y otros monoterpenos en grandes cantidades, principalmente en la atmósfera. El limoneno se utiliza como disolvente para desengrasar los metales antes de la pintura industrial, para la limpieza en la industria electrónica y de la imprenta, y como disolvente en la pintura. Se emplea asimismo como aditivo de sabor y aroma en alimentos, productos de limpieza de uso doméstico y perfumes.

El limoneno es un irritante de la piel, tanto en animales de experimentación como en seres humanos. Se ha comprobado que en los conejos el *d*-limoneno irrita los ojos. Estudios efectuados con cobayos han revelado que el *d*-limoneno oxidado por el aire, pero no propiamente el *d*-limoneno, produce dermatitis de contacto. Como el *d*-limoneno y el *l*-limoneno son enantiómeros, lo anterior podría ser cierto también para el *l*-limoneno y el dipenteno (la forma racémica). La manipulación y la pureza de la sustancia química, y posiblemente la adición de antioxidantes, pueden influir por tanto de manera crucial en la alergenicidad del limoneno.

El órgano crítico en los animales (excepto en las ratas macho), tras la administración oral o intraperitoneal de la sustancia, es el hígado. No se sabe de estudios en que los animales de experimentación hayan sido expuestos al limoneno por inhalación. La exposición al limoneno altera la cantidad y la actividad de distintas enzimas hepáticas, el peso del hígado, los niveles de colesterol y la secreción biliar. Se han observado esos cambios en ratones, ratas y perros. No se dispone de suficientes datos para determinar cuál es el órgano crítico en el ser humano.

En las ratas macho, la exposición al *d*-limoneno causa lesiones y tumores renales. Se cree que una proteína específica de la rata macho, la "2: -globulina, cumple una función crucial en el desarrollo de lesiones renales tanto neoplásicas como no neoplásicas. Por consiguiente, esas lesiones renales se consideran sin importancia para la evaluación del riesgo en el ser humano. El *d*-limoneno se estudió en una batería de pruebas *in vitro* de corta duración demostrándose que no es genotóxico. No hay constancia de que el limoneno tenga efectos teratogénicos o embriotóxicos en ausencia de toxicidad materna. En general, el *d*-limoneno puede considerarse una sustancia química con una toxicidad bastante baja (salvo por sus propiedades irritantes y sensibilizantes).

Según los datos de que se dispone, los alimentos son la principal fuente de exposición al limoneno. El valor de orientación calculado para la ingestión de limoneno es de 0,1 mg por kg de peso corporal diarios. A los actuales niveles de exposición estimados, el limoneno presente en los productos alimenticios no parece constituir un riesgo significativo para la salud humana.

En la atmósfera, el limoneno y otros terpenos reaccionan rápidamente con los radicales hidroxilo y nitrato y con el ozono producidos fotoquímicamente. La oxidación de los terpenos como el limoneno contribuye a la formación de aerosoles y de niebla fotoquímica. En principio, en el suelo el limoneno tiene poca movilidad, y en el medio acuático se une fuertemente al sedimento. El limoneno es resistente a la hidrólisis. La biodegradación se produce en condiciones aerobias, pero no en condiciones anaerobias.

La vía de exposición al limoneno más probable en los organismos terrestres es el aire. Los pocos estudios realizados sobre especies terrestres (esto es, insectos) mediante exposición al vapor han revelado efectos en niveles de partes por millón. Las concentraciones ambientales medidas oscilan generalmente entre 0,1 y 2 ppm (0,6–11 : g/m³). En lugares contaminados, las concentraciones de limoneno en el suelo pueden superar los niveles por encima de los cuales aparecen efectos en los organismos que viven en el suelo (por

ejemplo, los gusanos). En el medio acuático, el limoneno presenta una elevada toxicidad aguda para los peces y para *Daphnia*. Las concentraciones de limoneno en aguas superficiales suelen ser mucho más bajas que los niveles de toxicidad aguda determinados experimentalmente, por lo que es probable que el limoneno plantee un bajo riesgo de efectos tóxicos agudos para los organismos acuáticos. No se conocen estudios sobre los efectos crónicos.