d-Limonene; CASRN 5989-27-5

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR d-Limonene

File First On-Line 12/01/1993

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	not evaluated	
Inhalation RfC (I.B.)	qualitative discussion	12/01/1993
Carcinogenicity Assessment (II.)	not evaluated	

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — d-Limonene
CASRN — 5989-27-5

Not available at this time.

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — d-Limonene
CASRN — 5989-27-5

The health effects data for d-limonene were reviewed by the U.S. EPA RfD/RfC Work Group and determined to be inadequate for the derivation of an inhalation RfC. The verification status for this chemical is currently NOT VERIFIABLE. For additional information on the health effects of this chemical, interested parties are referred to the documentation listed below.

NOT VERIFIABLE status indicates that the U.S. EPA RfD/RfC Work Group deemed the database at the time of review to be insufficient to derive an inhalation RfC according to the Interim Methods for Development of Inhalation Reference Concentrations (U.S. EPA, 1990). This status does not preclude the use of information in cited references for assessment by others.

d-Limonene (1-methyl-4-isopropenyl-1-cyclohexene) is a liquid with a lemonlike odor. It is a major constituent in several citrus oils (orange, lemon, mandarin, lime, and grapefruit) and is present in a number of other essential oils, as well. d-Limonene is included on the Food and Drug Administration's (FDA's) Generally Recognized as Safe List and is approved for use by the FDA as a food additive (Opdyke, 1975). d-Limonene has a boiling point of 176 C and a vapor pressure of <3 mmHg at 14 C. d-Limonene is used primarily as a flavor and fragrance ingredient.

No information is available on the health effects of inhalation exposure to d-limonene in humans, and no long-term inhalation studies have been conducted in laboratory animals. NTP (1990) conducted a series of studies that investigated the toxicity of d-limonene (>99% pure) in both Fischer 344/N rats and B6C3F1 mice. In the first of the preliminary range-finding studies, doses ranging from 413-6600 mg/kg/day were administered by gavage in corn oil to five animals/species/sex/dose for 5 days/week for 16 days. All but 2/20 rats and 1/20 mice that were administered 3300 and 6600 mg/kg/day died. Body weight gain was reduced at 1650 mg/kg/day. No compound-related signs of toxicity were observed in those animals administered <1650 mg/kg/day.

In the 13-week study, 10 animals/species/sex/dose were administered 0, 150, 300, 600, 1200, or 2400 mg/kg/day (rats) or 0, 125, 250, 500, 1000, or 2000 mg/kg/day (mice) d-limonene by gavage in corn oil for 5 days/week. Survival was reduced in the high-dose rats, and body weight gain decreased in a dose-related fashion in the male rats starting at 600 mg/kg/day. Male rats that were administered 1200 or 2400 mg/kg/day exhibited rough hair coats, lethargy, and excessive lacrimation. The only compound-related effect noted in rats was nephropathy in males. Survival also was reduced slightly in the mice that received 2000 mg/kg/day, and decreased body weight gain was observed in those male mice that were administered the two highest doses of d-limonene. Aside from the observation of rough hair coats and decreased activity in the mice receiving 1000 and 2000 mg/kg/day, no other compound-related signs of toxicity or lesions were noted.

In the 2-year study, 50 animals/species/sex/dose were administered 0, 75, or 150 mg/kg/day (male rats); 0, 300, or 600 mg/kg/day (female rats); 0, 250, or 500 mg/kg/day (male mice); or 0, 500, or 1000 mg/kg/day (female mice) d- limonene by gavage in corn oil once a day for 5 days/week. The survival of the female rats administered 600 mg/kg/day was significantly lower than that of the vehicle controls.

The only microscopic evidence of compound-related toxicity noted in the rats was nephropathy in the males. d-Limonene is one of a diverse group of hydrocarbons that has been shown to induce a unique syndrome of nephropathy in male rats following subchronic or chronic exposure. Based on a review of the literature concerning this effect (U.S. EPA, 1991), EPA's Risk Assessment Forum concluded that nephropathy in male rats that is associated with alpha- 2u-globulin accumulation in hyaline droplets is not an appropriate endpoint to determine noncancer effects potentially occurring in humans.

Female mice exposed to 1000 mg/kg/day d-limonene exhibited 5-15% lower mean body weights than their respective vehicle controls after week 28 of the study. No compound-related clinical signs of toxicity were noted in either sex. An increased incidence of multinucleated hepatocytes and cytomegaly was observed in the high-dose male mice but not in female mice. Based on the occurrence of these liver lesions, a NOAEL of 250 mg/kg/day and a LOAEL of 500 mg/kg/day can be estimated from this study in mice.

Nephrotoxicity, consisting of granular casts characteristic of alpha-2u- globulin-mediated nephropathy, was observed in male Sprague-Dawley rats administered 277, 554, or 1385 mg/kg/day d-limonene daily by gavage in 1% Tween 80 for 6 months (Tsuji et al., 1975). These lesions were not observed in the female rats similarly exposed.

The developmental toxicity of d-limonene has been investigated in mice and rabbits. In the mouse study, 15 pregnant ICR mice/group were administered 0, 591, or 2363 mg/kg/day d-limonene by gavage on gestation days 7-12 (Kodama et al., 1977a). Maternal toxicity (significant reduction in body weight) and developmental toxicity (significant increase in the number of fetuses with skeletal abnormalities, including lumbar ribs, fused ribs, and delayed ossification of several bones in the paws) were observed in the animals administered 2363 mg/kg/day. No maternal or fetal effects were observed at the low dose. This study is limited in that an inadequate number of animals was used, only two doses were tested, and dosing did not continue throughout the entire period of organogenesis.

In the rabbit study, 10-18 pregnant Japanese white rabbits were administered 0, 250, 500, or 1000 mg/kg/day d-limonene by gavage on gestation days 6-18 (Kodama et al., 1977b). Exposure of does to 500 or 1000 mg/kg/day resulted in maternal toxicity. There were significant reductions in food consumption and body weight at both doses, and death also occurred in the 1000-

mg/kg/day group. Developmental toxicity was not observed at any dose. This study is limited by the small sample size.

No reproductive toxicity studies have been conducted on d-limonene. Igimi et al. (1974) studied the metabolism of d-limonene after oral administration and found that about 65% of the dose was recovered in urine, feces, and expired carbon dioxide, suggesting that the majority of an oral dose is absorbed. Although it is possible that an inhaled dose would also be largely absorbed, there is no information on inhalation exposures.

An RfC cannot be derived because of the lack of information on possible respiratory tract effects and the limited pharmacokinetic data on which to base a route extrapolation.

Igimi, H., M. Nishimura, R. Kodama, and H. Ide. 1974. Studies on the metabolism of d-limonene (p-mentha-1,8-diene). I. The absorption, distribution, and excretion of d-limonene in rats. Xenobiotica. 4(2): 77-84.

Kodama, R., A. Okubo, E. Araki, K. Noda, H. Ide, and T. Ikeda. 1977a. Studies on d-limonene as a gallstone solubilizer. (VII). Effects on development of mouse fetuses and offsprings. Oyo Yakuri. 13(6): 863-873.

Kodama, R., A. Okubo, K. Sato et al. 1977b. Studies on d-limonene as a gallstone solubilizer. (IX). Effects on development of mouse fetuses and offsprings. Oyo Yakuri. 13(6): 885-898.

NTP (National Toxicology Program). 1990. Toxicology and carcinogenesis studies of d-limonene (CAS No. 5989-27-5) in F344/N rats and B6C3F1 mice (gavage studies). NTP Technical Report Series No. 347, NIH PB No. 90-2802, U.S. DHHS, National Institutes of Health.

Opdyke, D.J.L. 1975. Special Issue II: Monographs on fragrance raw materials. Food Cosmet. Toxicol. 13: 825-826.

Tsuji, M., Y. Fujisaki, Y. Arikawa et al. 1975. Studies on d-limonene as a gallstone solubilizer. (III). Chronic toxicity in rats. Oyo Yakuri. 9(3): 403-412.

U.S. EPA. 1990. Interim Methods for Development of Inhalation Reference Concentrations (External Review Draft). Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Research Triangle Park, NC. EPA/600/8-90/066A.

U.S. EPA. 1991. Alpha2u-globulin: Association with chemically-induced renal toxicity and neoplasia in the male rat. Washington, DC. EPA/625/3-91/019F.

Agency Work Group Review — 09/23/1993

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfC for d-Limonene conducted in September 2002 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

EPA Contacts:

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — d-Limonene
CASRN — 5989-27-5

This substance/agent has not undergone a complete evaluation and determination under US EPA's IRIS program for evidence of human carcinogenic potential.

III. [reserved]
IV. [reserved]
V. [reserved]

VI. Bibliography

Substance Name — d-Limonene
CASRN — 5989-27-5

VI.A. Oral RfD References

None

VI.B. Inhalation RfC References

Igimi, H., M. Nishimura, R. Kodama, and H. Ide. 1974. Studies on the metabolism of d-limonene (p-mentha-1,8-diene). I. The absorption, distribution, and excretion of d-limonene in rats. Xenobiotica. 4(2): 77-84.

Kodama, R., A. Okubo, E. Araki, K. Noda, H. Ide, and T. Ikeda. 1977a. Studies on d-limonene as a gallstone solubilizer. (VII). Effects on development of mouse fetuses and offsprings. Oyo Yakuri. 13(6): 863-873.

Kodama, R., A. Okubo, K. Sato et al. 1977b. Studies on d-limonene as a gallstone solubilizer. (IX). Effects on development of mouse fetuses and offsprings. Oyo Yakuri. 13(6): 885-898.

NTP (National Toxicology Program). 1990. Toxicology and carcinogenesis studies of d-limonene (CAS No. 5989-27-5) in F344/N rats and B6C3F1 mice (gavage studies). NTP Technical Report Series No. 347, NIH PB No. 90-2802, U.S. DHHS, National Institutes of Health.

Opdyke, D.J.L. 1975. Special Issue II: Monographs on fragrance raw materials. Food Cosmet. Toxicol. 13: 825-826.

Tsuji, M., Y. Fujisaki, Y. Arikawa et al. 1975. Studies on d-limonene as a gallstone solubilizer. (III). Chronic toxicity in rats. Oyo Yakuri. 9(3): 403-412.

U.S. EPA. 1990. Interim Methods for Development of Inhalation Reference Concentrations (External Review Draft). Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Research Triangle Park, NC. EPA/600/8-90/066A.

U.S. EPA. 1991. Alpha2u-globulin: Association with chemically-induced renal toxicity and neoplasia in the male rat. Washington, DC. EPA/625/3-91/019F.

VI.C. Carcinogenicity Assessment References

None

VII. Revision History

Substance Name — d-Limonene
CASRN — 5989-27-5

Date	Section	Description
12/01/1993	I.B.	Inhalation RfC discussion on-line
12/03/2002	I.B.	Screening-Level Literature Review Findings message has been added.

VIII. Synonyms

Substance Name — d-Limonene
CASRN — 5989-27-5
Last Revised — 11/01/1993

- 5989-27-5
- Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)- (9CI)
- D-Limonene
- (+)-Limonene
- (+)-P-MENTHA-1,8-DIENE
- (R)-(+)-LIMONENE
- (R)-1-Methyl-4-(1-methylethenyl)cyclohexene
- AI3-15191
- Carvene
- CCRIS 671
- D-(+)-LIMONENE
- d-LIMONENO [Spanish]
- HSDB 4186
- NCI-C55572
- p-Mentha-1,8-diene, (R)-(+)-
- Refchole