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SAFETY EVALUATION AND RISK ASSESSMENT OF *D*-LIMONENE

Young Woo Kim[1], Min Ji Kim[1], Bu Young Chung[1], Du Yeon Bang[1], Seong Kwang Lim[1], Seul Min Choi[1], Duck Soo Lim[1], Myung Chan Cho[1], Kyungsil Yoon[2], Hyung Sik Kim[3], Kyu Bong Kim[4], You Sun Kim[5], Seung Jun Kwack[6], Byung-Mu Lee[1]

[1]Division of Toxicology, College of Pharmacy, Sungkyunkwan University, Suwon, South Korea
[2]Research Institute, National Cancer Center, Goyang, South Korea
[3]Molecular Toxicology, College of Pharmacy, Sungkyunkwan University, Suwon, South Korea
[4]College of Pharmacy, Dankook University, Chungnam, South Korea
[5]Foundation of Korea Cosmetic Industry Institute, Osan City, South Korea
[6]Department of Biochemistry and Health Science, College of Natural Sciences, Changwon National University, Changwon, South Korea

***d*-Limonene, a major constituent of citrus oils, is a monoterpene widely used as a flavor/fragrance additive in cosmetics, foods, and industrial solvents as it possesses a pleasant lemon-like odor. *d*-Limonene has been designated as a chemical with low toxicity based upon lethal dose (LD50) and repeated-dose toxicity studies when administered orally to animals. However, skin irritation or sensitizing potential was reported following widespread use of this agent in various consumer products. In experimental animals and humans, oxidation products or metabolites of *d*-limonene were shown to act as skin irritants. Carcinogenic effects have also been observed in male rats, but the mode of action (MOA) is considered irrelevant for humans as the protein α_{2u}-globulin responsible for this effect in rodents is absent in humans. Thus, the liver was identified as a critical target organ following oral administration of *d*-limonene. Other than the adverse dermal effects noted in humans, other notable toxic effects of *d*-limonene have not been reported. The reference dose (RfD), the no-observed-adverse-effect level (NOAEL), and the systemic exposure dose (SED) were determined and found to be 2.5 mg/kg/d, 250 mg/kg//d, and 1.48 mg/kg/d, respectively. Consequently, the margin of exposure (MOE = NOAEL/SED) of 169 was derived based upon the data, and the hazard index (HI = SED/RfD) for *d*-limonene is 0.592. Taking into consideration conservative estimation, *d*-limonene appears to exert no serious risk for human exposure. Based on adverse effects and risk assessments, *d*-limonene may be regarded as a safe ingredient. However, the potential occurrence of skin irritation necessitates regulation of this chemical as an ingredient in cosmetics. In conclusion, the use of *d*-limonene in cosmetics is safe under the current regulatory guidelines for cosmetics.**

Limonene (also referred to as *d*-limonene, *l*-limonene, and *dl*-limonene), a monocyclic monoterpene, is a major constituent of citrus oils such as those found in oranges, grapefruits, and lemons and possesses a lemon-like odor (U.S. EPA, 1994). Limonene has been widely used as a flavor/fragrance additive in perfumes, soaps, pharmaceuticals, and foods because of its pleasant citrus fragrance (Sun, 2007). Limonene was detected in approximately 71% of 300 fragrance products in the Netherlands (de Groot and Frosch, 1997). Limonene has also be used as an active or inert ingredient in pesticides, solvents, degreasers, and cleaning agents (U.S. EPA, 2004; Yoon et al., 2010).

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Address correspondence to Dr. Byung-Mu Lee, Division of Toxicology, College of Pharmacy, Sungkyunkwan University, Chunchun-dong 300, Changan-ku, Suwon, Gyeonggi-Do, 440-746, South Korea. E-mail: bmlee@skku.edu

d-Limonene is considered to be a natural substance possessing low toxicity (later in Table 2, oral LD 50 values 5–6 g/kg) and is registered in the Code of Federal Regulation (CFR) as a generally recognized as safe (GRAS) substance for synthetic flavorings (U.S. FDA, 2012). However, based upon the observation that *d*-limonene produces dermal irritation, the purposes of this review were to evaluate the safety of *d*-limonene as an ingredient in cosmetics and consumer products and to provide a comprehensive risk assessment.

PHYSICAL AND CHEMICAL PROPERTIES

Limonene is a colorless liquid at room temperature with a citrus odor (Matura et al., 2002). Figure 1 shows the chemical formula of limonene. The chemical exists as two optical isomers, *d*-limonene (found in oranges) and *l*-limonene (present in lemons), and the racemic mixture dipentene (*dl*-limonene) (WHO, 1998). These isomers are found in different amounts and ratios in various plants. The predominant isomeric form of limonene is *d*-limonene, which is present in nearly 98% of all citrus oils (Christensson et al., 2009). Limonene is readily oxidized in air during handling and storage, and its oxidation products are known to be allergenic (Matura et al., 2002; WHO, 1998). The limonene oxidation products are R-(+)-limonene oxide, R-(–)-carvone, and R-(+)-limonene 2-hydroperoxide. The physical and chemical properties of *d*-limonene are presented in Table 1.

USES

Limonene is widely used in soaps and perfumes because of its lemon-like odor, which is derived from its major presence in citrus oils (U.S. EPA, 1994). In addition, limonene has been used as a flavor/fragrance additive in foods and in household cleaning products. Limonene is also used in pesticides, insect repellents, and dog/cat repellents (U.S. EPA, 1994). Because of its solvent properties for cholesterol, *d*-limonene has been used clinically to dissolve cholesterol-containing gallstones (Igimi et al., 1991). Because of its capability to neutralize gastric acid, *d*-limonene has been used to relieve heartburn and gastroesophageal reflux (Sun, 2007). The chemotherapeutic activity of *d*-limonene was also studied in human prostate cancer cell lines and animal models (Broitman et al., 1996; Crowell et al., 1992; Gould, 1997; Rabi and Bishayee, 2009). Phase I/II clinical trials of *d*-limonene were performed for chemical safety and pharmacokinetics in cancer patients at clinical therapeutic doses (Gould, 1997; Vigushin et al., 1998). At present, *d*-limonene is still undergoing further trials as a chemotherapeutic agent. It is worth noting that limonene is increasingly being used as a substitute for chlorinated hydrocarbons, chlorofluorocarbons (CFC), and other solvents (WHO, 1998), as it is considered to exert less toxicity.

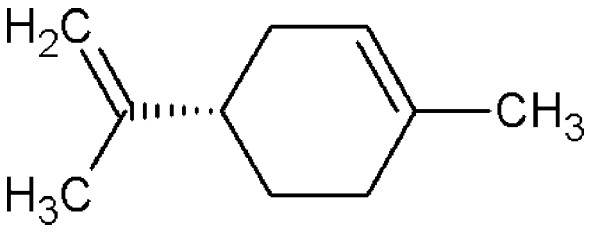

FIGURE 1. Chemical structure of *d*-limonene ((*R*)-enantiomer).

PRODUCTION

The annual global production of *d*-limonene and orange oil/essence oils (95% *d*-limonene) in 1991 was approximately 45 kilotonnes (1 kT = 1,000 tonnes). The production volume in Japan was approximately 40 kT in 1993 (WHO, 1998).

EXPOSURE DATA

Environment

Limonene occurs naturally in citrus and other fruits, vegetables, meats, and spices (Matura et al., 2002). Limonene is categorized as practically nontoxic to freshwater fish and mildly toxic to freshwater invertebrates on an acute excess quantity basis (U.S. EPA, 1994). Environmental concentrations of

TABLE 1. Physical and Chemical Properties of *d*-Limonene

Properties	Value	Reference
CAS number	5989-27-5	NTP (1990)
EINECS number	227-813-5	
RTECS number	GW6360000	WHO (1998)
IUPAC name	1-Methyl-4-(1methylethenyl)-cyclohexene	
INCI name	Limonene	
Molecular formula	C10H16	
Synonyms	*R*-(+)-limonene, (+)-limonene, *R*-limonene	Larsen et al. (2000)
Molar mass	136.24 g/mol	U.S. NOAA (2011)
Melting point	>–74.35°C	
Boiling point	>176°C	
Specific gravity (20°C)	0.84	
Flash point	48°C	WHO (1998)
Density (20°C)	0.8411 g/cm^3	
Vapor pressure (20°C)	190 Pa	
Water solubility (25°C)	13.8 mg/L	
Autoflammability	237°C	IPCS (2005)
Octanol/water partition coefficient (log *K*ow)	4.45	

Note. CAS: Chemical Abstracts Service; EINECS: European Inventory of Existing Commercial chemical Substances; RTECS: Registry of Toxic Effects of Chemical Substances; IUPAC: International Union of Pure and Applied Chemistry; INCI: International Nomenclature of Cosmetic Ingredients.

limonene generally range from 0.1 to 2 ppb (0.6–11 g/m^3) (WHO, 1998). Mean emission rates for limonene from various plant species in California's Central Valley ranged from 0.4 to 2.5 mg/g dry leaf weight per hour (Arey et al., 1991). Global annual emissions of biogenic monoterpenes ranged from 147 to 827 million tonnes (Fehsenfeld et al., 1992). Concentrations of limonene and other monoterpenes in air vary widely. Recorded concentrations in rural areas depend upon many factors, including type of vegetation, temperature, and time of the day/year (Strömvall, 1992). Biogenic monoterpene emissions were presumed to be lower in late autumn and winter months than in summer (Altshuller, 1983). Between 1979 and 1992, measured concentrations of limonene in air in rural forest areas in Europe, Canada, the United States, Nepal, and Japan ranged from 1.6×10^{-4} to 2.2 ppb (0.9 ng/m^3 to 12.2 g/m^3) (Ciccioli et al., 1993; Helmig and Arey, 1992; Hutte et al., 1984; Jüttner, 1988; Roberts et al., 1985; Shaw et al., 1983). These data showed that estimated concentrations of limonene in air in rural areas ranged from 0.1 to 0.2 ppb (0.6–1.1 g/m^3).

Limonene has been detected in water, sediments, and soils. Sauer (1981) collected water samples from the Gulf of Mexico and reported limonene concentrations ranging from 2 to 40 ng/L. In addition, Desideri et al. (1991) found limonene in Terra Nova Bay, Antarctica. McCreary et al. (1983) measured a polluted area in Florida at a former site for charcoal and pine tar production and detected limonene concentrations up to 920 g/g in soil and from 1 to 130 g/L in groundwater.

Human Exposure

Exposure to *d*—limonene may occur through the use of cosmetics and certain pesticide products, including insect repellents and dog/cat repellents. In addition, exposure may occur naturally through different foods or through Food and Drug Administration (FDA)–approved GRAS uses such as additives in food products, soaps, and perfumes (U.S. EPA, 2004; U.S. FDA, 2011). Exposure to limonene via indoor and outdoor air for the general population is estimated to be 10 and 0.1g/kg/d, respectively, which is based upon some assumptions in a study from Los Angeles, including daily inhalation volume for adults of 22 m^3, mean body weight for males and females of 64 kg, the assumption that 4 out of 24 h are spent outdoors (IPCS, 1994), and arithmetic mean limonene levels in indoor and

outdoor air of 0.04 and 0.002 mg/m^3, respectively (Wallace et al., 1991). The estimated exposure to limonene from occupational sources was calculated on the same basis as that for indoor and outdoor air by assuming that 8 out of 24 h are spent in the workplace each day and the air concentration is 150 mg/m^3, which is the occupational exposure limit in Sweden (NBOSH, 1993). The intake of limonene for occupational exposure limit was estimated to be 17 mg/kg/d.

TOXICOLOGICAL EVALUATION

Toxicokinetics (Absorption, Distribution, Metabolism, Excretion; ADME)

Absorption Following oral administration, absorption of *d*-limonene is rapid, and almost complete in the gastrointestinal tract (GIT) in humans and animals (Crowell et al., 1992; Igimi et al., 1974; Vigushin et al., 1998). In humans, 52–83% of the dose is excreted in urine within 48 h after ingestion of 1.6 g [^{14}C]-*d*-limonene (Kodama et al., 1976). The oral bioavailability of *d*-limonene is 43% (Chen et al., 1998). Dermal absorption of [^{3}H]-*dl*-limonene in shaved mice through bathing water is rapid, and the maximal level is reached in 10 min (Schäfer and Schäfer, 1982).

Distribution Limonene is distributed to different tissues in the body, and clearance from blood is 1.1 L/kg/h in males following inhalation exposure for 2 h to *d*-limonene at a concentration of 450 mg/m^3 (Falk–Filipsson et al., 1993). In Wistar rats, tissue distribution of radioactivity is initially high in the liver, kidneys, and blood after oral administration of [^{14}C]-*d*-limonene, and negligible amounts of radioactivity are found after 48 h (Igimi et al., 1974). In Wistar–Furth rats, limonene and its metabolites appear within 20 min after oral administration (Crowell et al., 1992). The half-life of limonene in both Wistar–Furth rats and humans was estimated to be 12–24 h, and excretion occurred primarily through the urine. For rats, there are gender-related variations (Webb et al., 1989). The concentration of *d*-limonene is approximately threefold higher in male F344 rats than females, and approximately 40% of *d*-limonene is reversibly bound to α_{2u}-globulin, a protein specifically detected in male rats (Lehman-McKeeman and Caudill, 1992).

Metabolism The metabolism of *d*-limonene in mammals is illustrated in Figure 2. Limonene is metabolized to oxygenated metabolites in rats and humans. In rats, the two major serum metabolites of limonene are perillic acid and dihydroperillic acid. Humans produce these two serum metabolites as well as limonene-1,2-diol (Crowell et al., 1992). The biotransformation of *d*-limonene was examined in various species, and several possible metabolic pathways were identified. Approximately 25–30% of an oral dose of *d*-limonene in humans is found in the urine as *d*-limonene-8,9-diol and its glucuronide. Further, approximately 7–11% is eliminated as perillic acid [4-(1-methylethenyl)-1-cyclohexene-1-carboxylic acid] and its metabolites (Kodama et al., 1976). *d*-Limonene-8,9-diol may be formed via *d*-limonene-8,9-epoxide (Watabe et al., 1981). Other reported pathways of limonene metabolism involve ring hydroxylation and oxidation of the methyl group (Kodama et al., 1976). Upon oxidation, limonene may be transformed into carvone, carveol, and limonene oxide.

Poon et al. (1996) examined the toxicokinetics of *d*-limonene in two female patients with breast cancer and one male patient with colorectal cancer. The patients received 0.5–12 g/m^2 body surface area per day orally for 21 d, and plasma and urine samples were collected on d 1 and 21. By liquid chromatography–mass spectrometry (LC/MS) and nuclear magnetic resonance (NMR) spectrometry, five major metabolites were detected in the plasma: limonene-1,2-diol, limonene-8,9-diol, perillic acid, an isomer of perillic acid, and dihydroperillic acid. The urinary metabolites were composed of the glucuronides of the two isomers of perillic acid, limonene-8,9-diol, and monohydroxylated limonene. In a Phase I clinical trial of orally administered *d*-limonene, 17 women and 15 men between the ages of 35 and 78 (median = 57) with solid

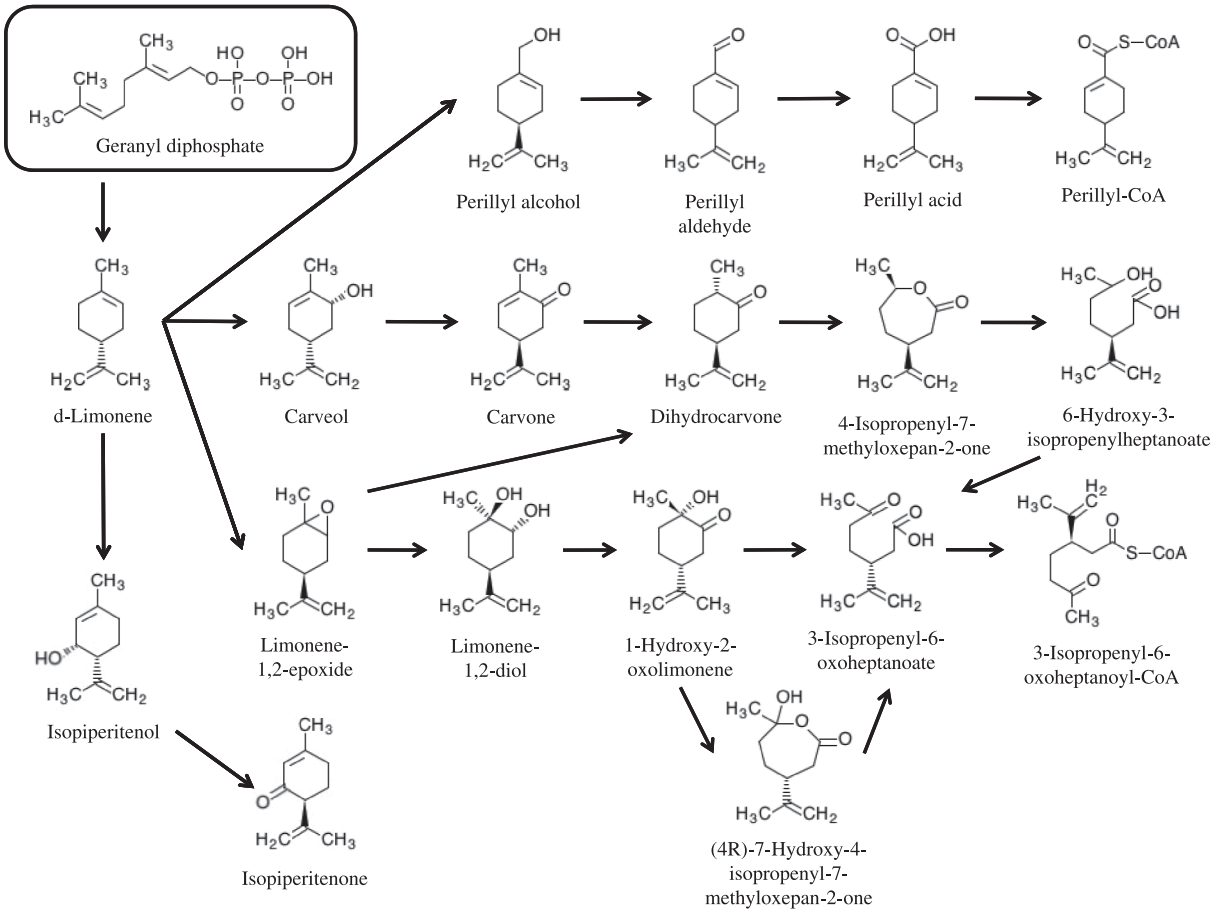

FIGURE 2. Metabolic pathway of *d*-limonene. *d*-Limonene is synthesized from geranyl diphosphate (GPP) in several plants or microorganisms. Biotransformation of *d*-limonene is demonstrated here. (Data from Bicas et al., 2008; Duetz et al., 2003; KEGG, 2012; van der Werf et al., 1997.)

tumors received an average of three treatment cycles at doses ranging from 0.5 to 12 g/m^2 body surface area (Vigushin et al., 1998). The predominant metabolites were perillic acid (21–71 μmol/L), dihydroperillic acid (17–28 μmol/L), limonene-1,2-diol (10–21 μmol/L), uroterpinol (14–45 μmol/L), and an isomer of perillic acid. There was no accumulation of parent compound or metabolites after repetitive doses for 21 d. These results are consistent with the findings of previous studies (Kodama et al., 1976; Watabe et al, 1981; Crowell et al., 1992).

Excretion The half-life ($t_{1/2}$) of *d*-limonene in humans is relatively short, ranging from 12 to 24 h (Crowell et al., 1992), and excretion is primarily through the urine (Igimi et al., 1974; Kodama et al., 1976). Following inhalation exposure of volunteers to *d*-limonene at 450 mg/m^3 for 2 h, three phases of elimination were observed in blood, and the half-lives were approximately 3, 33, and 750 min, respectively (Falk–Filipsson et al., 1993). Approximately 1% of the amount absorbed was eliminated unchanged in exhaled air, whereas approximately 0.003% was eliminated unchanged in urine. When male volunteers were administered 1.6 g [^{14}C]-*d*-limonene, 50–80% of the radioactivity was eliminated in urine within 2 d (Kodama et al., 1976). It is of interest that limonene was detected but not quantified in breast milk of nonoccupationally exposed mothers (Pellizzari et al., 1982).

ADVERSE EFFECTS

General Toxicity

Limonene is a dermal irritant at high concentrations, but is not allergenic (Okabe et al., 1990). Limonene is classified as a skin sensitizer in Europe because of the formation of allergenic oxidation products. As a potential irritant, *d*-limonene was found to produce purpuric rash following dermal exposure (Falk et al., 1991) and skin irritation in rats and rabbits (Okabe et al., 1990). In addition, sensitization was shown following inhalation using Dunkin–Hartley albino guinea pigs (Karlberg et al., 1991). Further, Karlberg et al. (1991) demonstrated that *R*-(+)-limonene forms allergenic oxidation products such as limonene oxide, limonene hydroperoxides, and *R*-(–)-carvone during handling and storage. The oxidized *R*-limonene has more irritating and sensitizing potential than the nonoxidized one (Christensson et al., 2008, 2009). However, the hydroperoxides of *R*-(+)-limonene are unstable and degrade readily to secondary oxidation products. The hydroperoxides are reduced to corresponding alcohols in the presence of α-tocopherol acetate, an antioxidant often incorporated into petrolatum to be used for patch-test preparations to prevent oxidation (Nilsson et al., 1999).

A secondary adverse effect may also be possible following exposure to limonene involving metabolic reactions. Walser et al. (2007) suggested that formaldehyde is formed when limonene reacts with ozone. Other reaction products generated by the reaction of limonene with ambient ozone or free radicals may subsequently produce secondary organic aerosols either by multiphase oxidation or by photooxidation and thus be responsible for the irritability ascribed to limonene (EC, 2006; Maksymiuk et al., 2009; Kim et al., 2012). Rolseth et al. (2002) in vitro showed that *d*-limonene was toxic to human lung cells. In contrast, Wolkoff et al. (2008) indicated that ultrafine particles generated during oxidation process of limonene may not be causative agents for sensory effects of ozone-initiated limonene metabolism in the pulmonary airways.

Acute Studies

The acute toxicity of *d*-limonene to rodents is quite low following oral, intraperitoneal (ip), subcutaneous (sc), or intravenous (iv) administration based on the LD_{50} value as indicated in Table 2 (Tsuji et al., 1974, 1975a). Oral LD_{50} values of rodents were determined to be 5 g/kg in rats and 6 g/kg in mice (Tsuji et al., 1974). Opdyke (1979) reported that the LD_{50} value of *d*-limonene for rabbits was at least 5 g/kg via dermal application.

The National Toxicology Program (NTP, 1990) conducted an acute oral study of *d*-limonene using F344/N rats and B6C3F1 mice, 5 animals per group per gender for 16 d (Table 2). All animals received *d*-limonene orally at the dose of 0, 413, 825, 1650, 3300, or 6600 mg/kg/d. The no-observed-adverse-effect level (NOAEL) of *d*-limonene was found to be 1650 mg/kg/d in both species. Lethality was noted at 3300 mg/kg/d in both species.

Subchronic Studies

The oral administration of *d*-limonene to Wistar rats at a dose of 400 mg/kg body weight for 30 d resulted in a 20–30% increase in the quantity and activity of different liver enzymes (cytochrome b5, aminopyrine demethylase, and aniline hydroxylase), an elevation in relative liver weight, and a decrease in cholesterol levels (Ariyoshi et al., 1975) (Table 2). Kanerva et al. (1987) administered limonene orally, daily for 26 d, a dose of 75, 150, or 300 mg/kg to male F344 rats. An increase in relative kidney and liver weight occurred at 300 mg/kg. At 75 mg/kg/d hyaline droplet formation was noted only in kidneys of chemical-treated male rats. The NOAEL for liver was determined to be 150 mg/kg/d, while the kidney lowest-observed-adverse-effect level (LOAEL) was 75 mg/kg/d. However, it is worth noting that this renal effect is not relevant for humans. The administration of *d*-limonene (0, 2, 5, 10, 30, or 75 mg/kg/d) by gavage to groups of 10 male F344 rats 5 d/wk for 13 wk led to the pathological formation of granular casts in the outer zone of the renal medulla (Webb et al., 1989).

TABLE 2. Acute, Subchronic, or Chronic Toxicity of d-Limonene

Studies	Species	Route	Time	Dose	Results	Reference
LD50	Rat (SD)	Oral			5 g/kg	Tsuji et al. (1974)
	Mouse	Oral			6 g/kg	
	Rat	ip			3.6 g/kg for male, 4.5 g/kg for female	
	Rat	iv			0.125 g/kg for male, 0.11 g/kg for female	Tsuji et al. (1975a)
	Mouse	sc			>41.5 g/kg	
	Rabbit	Dermal			>5 g/kg	Opdyke (1979)
Acute	Mouse (B6C3F1)	Oral	16 d	0, 413, 825, 1650, 3300, or 6600 mg/kg/d	NOAEL: 1650 mg/kg/d LOAEL: 3300 mg/kg/d Death at 3300 and 6600 mg/kg/d No compound-related clinical signs observed in mice in the 1650 mg/kg dose group No compound-related histopathologic effects	NTP (1990)
	Rat (F344/N)				NOAEL: 1650 mg/kg/d LOAEL: 3300 mg/kg/d Death at 3300 and 6600 mg/kg/d No clinical signs in the 1650 mg/kg dose group or lower. No compound-related histopathologic effects	
Subchronic	Male rat (Wistar)	Oral	30 d	400 mg/kg	20–30% increase in amount and activity of liver enzymes Increase in relative liver weight Decrease in cholesterol levels	Ariyoshi et al. (1975)
Subchronic	Male rat (F344)	Oral	26 d	0, 75, 150, or 300 mg/kg/d	NOAEL: undetermined (kidney) 150 mg/kg/d (liver) LOAEL: 75 mg/kg/d (kidney) 300 mg/kg/d (liver) Increased relative kidney and liver weight in 300-mg/kg/d group Kidneys showed dose-related hyaline droplet formation, granular casts in the outer zone of the medulla, and chronic nephrosis but no alterations in liver	Kanerva et al. (1987)
	Rat (F344)	Oral	10 male 5d/wk for 13 wk	0, 2, 5, 10, 30 or 75 mg/kg/d	Pathological formation of granular casts in the outer zone of the renal medulla NOEL = 5 mg/kg/d LOEL = 75 mg/kg/d NOAEL = 30 mg/kg/d	Webb et al. (1989)
	Mouse (B6C3F1)	Oral	13 wk	0, 125, 250, 500, 1,000 or 2,000 mg/kg/d	NOAEL: 500 mg/kg/d LOAEL: 1000 mg/kg/d Decrease in body weight and death Rough hair coats and decreased activity	NTP (1990)

(*Continued*)

TABLE 2. (*Continued*)

Studies	Species	Route	Time	Dose	Results	Reference
	Rat (F344/N)			0, 150, 300, 600, 1,200 or 2,400 mg/kg/d	NOAEL: undetermined in males, 600 mg/kg/d in females LOAEL: 150 mg/kg/d in males, 1200 mg/kg/d in females Males: nephropathy in the kidney at all doses Females: rough hair coats at 1200 mg/kg, lethargy, and excessive lacrimation	
Chronic	Dog	Oral	6 mo	0.4, 1.2, or 3.6 ml/kg/d	Vomiting and nausea Decreased body weight Decreased blood sugar and blood cholesterol Histopathologic lesions in kidney	Tsuji et al. (1975b)
	Rat (F344/N)	Oral	103 wk	Males: 0, 75, or 150 mg/kg/d Females: 0, 300, or 600 mg/kg/d	NOAEL: undetermined (males; kidney), 150 mg/kg/d (males; other than the kidney), 300 mg/kg/d in females LOAEL: 75 mg/kg/d (males: kidney), undetermined (males; other than the kidney), 600 mg/kg/d in females Males: effects on the kidney included dose-related increases in incidence of mineralization and epithelial hyperplasia even at the lowest dose tested No other effects at 150 mg/kg/d other than on kidney Decreases in survival rates for females at 600 mg/kg/d but no adverse effects at 300 mg/kg/d	NTP (1990)
Chronic	Mouse (B6C3F1)	Oral	103 wk	Males: 0, 250, or 500 mg/kg/d Females: 0, 500, or 1000 mg/kg/d	NOAEL: 250 mg/kg/d (male) 500 mg/kg/d (female) LOAEL: 500 mg/kg/d (male) 1,000 mg/kg/d (female) Males: liver cells with abnormal numbers of nuclei and cytomegaly at 500 mg/kg/d Females: decreases in survival rates and body weight (5–15%) for the 1,000 mg/kg group No treatment-related clinical signs for any group	NTP (1990)
	Dog (beagles)	Oral	6 mo	0, 100, or 1000 mg/kg/d	Sporadic diarrhea and emesis Dose-dependent increase in relative or absolute kidney weight in both male and female Increase serum cholesterol levels and alkaline phosphate activity at highest dose Absence of histopathological alterations	Webb et al. (1990)

The NTP (1990) conducted a subchronic oral study of limonene using F344/N rats and $B6C3F_1$ mice, 10 animals per group per gender for 13 wk. Results demonstrated a significant decrease in body weight, renal diseases, and tumors, as well as elevated rate of mortality at the highest dose tested. The no-observed-effect level (NOEL) based upon a histological examination of the kidney was estimated to be 5 mg/kg/d. The NOEL for increased liver and kidney weight was 75 mg/kg/d, the highest dose tested. The NOEL for the effects on the liver was 10 mg/kg, and the NOAEL for the effects on the liver was 30 mg/kg/d. A linear regression analysis revealed a dose-related trend in increased relative weight of kidney and liver at 30 and 75 mg/kg/d. No histopathological alterations were observed in liver in both these studies. The quantity and activity of different liver enzymes were not investigated, and therefore the rise in relative liver weight may have been due to enzyme induction.

Chronic Studies

The oral administration of *d*-limonene (0.4, 1.2, or 3.6 ml/kg/d) to dogs for 6 mo produced nausea and vomiting (Tsuji et al., 1975b) (Table 2). A 35% increase in serum alkaline phosphatase activity and cholesterol levels and numerical rise in total and relative liver weight in beagles occurred after the oral administration of *d*-limonene at a dose of 1.2 ml/kg/d for 6 mo (approximately 1000 mg/kg/d) (Webb et al., 1990).

The NTP (1990) conducted a 2-yr study in which *d*-limonene was administered orally 5 d/wk to groups of 50 F344/N rats (0, 75, or 150 mg/kg/d to males and 0, 300, or 600 mg/kg/d to females) and $B6C3F_1$ mice (0, 250, or 500 mg/kg/d to males and 0, 500, or 1000 mg/kg/d to females). After 28 wk, female mice exposed to 1000 mg/kg d-limonene daily showed a decrease in mean body weight of approximately 5–15% compared to controls. A numerical reduction in body weight was observed in rats in the high-dose group and male mice in the high-dose group. However, no apparent clinical symptoms related to the administration of *d*-limonene were noted. For female rats in the high-dose group, the survival rate decreased significantly after 39 wk, and microscopic evidence of compound-related nephropathy was noted for male rats as evidenced by α_{2u}-globulin accumulation in hyaline droplets. *d*-Limonene belongs to a group of hydrocarbons shown to induce a unique form of nephropathy in rats after subacute or chronic exposure, which is not an appropriate endpoint for humans because no this globulin does not accumulate in human kidney.

There was a dose-related increase in the incidence of hyperplasia and adenomas/adenocarcinomas in renal tubular cells of male rats, providing evidence of a carcinogenic effect of *d*-limonene. However, there was no evidence of carcinogenicity in female rats or male and female mice, indicating that the carcinogenic effect was gender and species specific. The carcinogenic response in the kidney of male rats has been linked to a unique renal perturbation involving α_{2u}-globulin. An increased incidence of multinucleated hepatocytes and cytomegaly was observed in male but not female mice. As a result of the presence of liver lesions, an NOAEL of 250 mg/kg/d and a lowest-observed-adverse-effect level (LOAEL) of 500 mg/kg/d were set for mice.

Carcinogenicity

Dietrich and Swenberg (1991) examined whether *d*-limonene would produce an increase in renal cell proliferation and exhibit promoting activity for the development of renal adenomas in male F344 rats (Table 3). The animals were administered orally *d*-limonene (150 mg/kg/d) as a promoter 5 d/wk for 30 wk. *N*-Ethyl-*N*-hydroxyethylnitrosamine (500 ppm) was used as an initiator in the drinking water for 2 wk. In addition, α_{2u}-globulin-deficient male rats were exposed in the same manner to determine whether α_{2u}-globulin, a urinary protein specific to male rats, would be required for *d*-limonene to produce these effects. *d*-Limonene treatment alone produced a significant rise in the number of atypical tubules

TABLE 3. Carcinogenic, Mutagenic, or Genotoxic Effects

Studies	Species	Route	Time	Dose	Results	Reference
Genotoxicity	*Salmonella typhimurium*			30 μmol/plate	Negative	Florin et al. (1980)
	Syrian hamster embryo cells				Negative No malignant transformation of cells	Pienta (1980)
	Salmonella typhimurium			0–20 μmol/plate	Negative	Watabe et al. (1981)
	Salmonella typhimurium			0–3333 μg/plate	Negative	Haworth et al. (1983)
	Salmonella typhimurium			10–500 μg/plate	Negative	Connor et al. (1985)
	Chinese hamster ovary cells				Negative No cytogenetic damage induced	Anderson et al. (1990)
	Chinese hamster ovary cells			15–162 μg/ml	Negative	NTP (1990)
	Mouse L5178Y cells			0.01–100 μl/ml	Negative No change in frequency of forward mutations at the TK+/- Locus	
	Rat tracheal epithelial cells			21.9 μ*M*	Inhibited transformation by BaP	Steele et al. (1990)
	Syrian hamster embryo cells		7 d	12.5, 25.0, or 50.0 μg/ml	Negative	Oshiro et al. (1998)
Mutagenicity	Mouse	ip injection	3 d	215 mg/kg/d	No evidence	Fahrig (1984)
Carcinogenicity	Rat (F344 and NBR)	Oral	5 d/wk for 30 wk	150 mg/kg/d	Increased number of atypical tubules and hyperplasia	Dietrich and Swenberg (1991)

and atypical hyperplasia in F344 rats compared to controls. However, no marked change in the incidence of tumors or preneoplastic lesions was observed in α_{2u}-globulin-deficient male rats exposed to *d*-limonene, whereas there was a 10-fold increase in the incidence of renal adenomas and atypical hyperplasia in F344 rats treated with *d*-limonene. In contrast, there was a significant decrease in the incidence of liver tumors in animals exposed to *N*-ethyl-*N*-hydroxyethylnitrosamine and *d*-limonene compared to only *N*-ethyl-*N*-hydroxyethylnitrosamine exposure. The International Agency for Research on Cancer (IARC) has classified *d*-limonene as Group 3, which indicates "not classifiable as to its carcinogenicity to humans" (IARC, 1999).

Genotoxicity

Genotoxicity studies (Table 3) on limonene provide no evidence that *d*-limonene or its metabolites are mutagenic. Limonene and its epoxides are not mutagenic when tested at concentrations of 0.3–3333 g/plate in assays in vitro using different strains of *Salmonella typhimurium* (Connor et al., 1985; Florin et al., 1980; Haworth et al., 1983; Watabe et al., 1981). Limonene did not produce forward mutation at the TK+/- locus in mouse L5178Y cells (NTP, 1990), induce cytogenetic damage to Chinese hamster ovary (CHO) cells (Anderson et al., 1990), or malignantly transform Syrian hamster embryo (SHE) cells (Pienta, 1980). In one in vitro study, following benzo[a]pyrene (BaP) treatment, *d*-limonene (21.9 mol/L) inhibited the formation of transformed cell colonies in the tracheal epithelium isolated from F344 rats (Steele et al., 1990). Fahrig (1984) provided no evidence of mutagenicity in an in vivo spot test with mice involving the intraperitoneal injection of limonene at 215 mg/kg/d on d 9–11 during gestation. Oshiro et al. (1998) conducted a Syrian hamster embryo (SHE) cell transformation assay for the investigation of *d*-limonene

toxicity and found no significant transformation frequency compared to the control.

Neurotoxicity

Effects of limonene exposure on the central nervous system (CNS) (Table 4) have been reported for animals, but it is difficult to interpret whether these results reflect general intoxication or a more direct effect of limonene. The oral administration of limonene to rats and mice was found to reduce motor activity (Tsuji et al., 1974), and similar effects were observed in $B6C3F_1$ mice treated orally with limonene at 1000 mg/kg/d for 13 wk (NTP, 1990). (+)-Limonene epoxide was found to produce sedative and anxiolytic-like effects in an acute study on male Swiss mice, which might involve an action on benzodiazepine-type receptors (de Almeida et al., 2012), On the other hand, *l*-limonene exerted antistress effects through $GABA_A$ receptor and inhibition of basal hypothalamic–pituitary–adrenal (HPA) activity in male Wistar rats under physical stress (Zhou et al., 2009). Nøjgaard et al. (2005) reported an adverse effect on human eyeblink frequency after treatment with *d*-limonene but the relevance remains unknown.

Immunotoxicity

There are few reported studies on immunotoxicity (Table 4). Evans et al. (1987) examined the immunological effects of *d*-limonene on B- and T-cell responses in BALB/*c* mice treated orally with *d*-limonene for 9 wk. In mice that were given keyhole limpet hemocyanin (KLH) before exposure to *d*-limonene there were suppressed primary and secondary anti-KLH responses, whereas those exposed to *d*-limonene before KLH administration showed significant increases in antibody- and mitogen-induced proliferative responses. In this study, *d*-limonene induced polyclonal activator action and polyclonal stimulation. However, Evans et al. (1987) did not determine the purity of *d*-limonene, and therefore oxidation products may have been the active substances,

TABLE 4. Toxicity of Central Nervous System (CNS), Immune System, and Skin

Studies	Species	Route	Time	Dose	Results	Reference
Neurotoxicity	Rat, mouse	Oral		3 ml	Decreased motor activity	Tsuji et al. (1974)
	Rat (Wistar)	Oral	7 d	0, 5, 25, or 50 mg/kg	Increased GABA concentration in the brain	Zhou et al. (2009)
	Mouse (Swiss)	Intraperitoneal	1 d	25, 50, or 75 mg/kg	Possible anxiolytic-like activity	de Almeida et al. (2012)
Immunotoxicity	Mouse (BALB/*c*)	Oral	Daily for 9 wk	0.1 ml	Suppression of primary and secondary anti-KLH responses Significant increases in antibody- and mitogen-induced proliferative responses	Evans et al. (1987)
	Rat (Wistar)	Subcutaneous	7 d	0.5, 2.5, or 5 mg	Negative for popliteal lymph node assay	Friedrich et al. (2007)
Dermal toxicity	Rabbit	Eye			Eye irritation	Tsuji et al. (1974)
	Himalayan white-spotted guinea pig	Patch tests			Moderate sensitization	Klecak et al. (1977)
	Mouse (BALB/*c*)	Dermal	Six times for 2 wk	0.1 ml	No sensitization	Maisey and Miller (1986)
	Rat/rabbit	Percutaneous			Low	Okabe et al. (1990)
	Dunkin–Hartley albino guinea pig	Dermal	Four times for 3 wk	0.1 ml	Only oxidation products of *d*-limonene produced sensitization	Karlberg et al. (1991)
	Rabbit (albino)	Dermal	<4 h	0.5 g	Primary irritation index 3.25–3.56 out of 8	Bagley et al. (1996)

such as Δ3-carene, pinene (a,b), myrcene, and sabinene. Friedrich et al. (2007) utilized the popliteal lymph node assay (PLNA), a screening test for detecting whether chemicals produce allergic and autoimmune-like reactions in humans, and found negative results for limonene.

Dermal Toxicity

d-Limonene is considered a skin irritant (Table 4). The skin irritation potential of limonene in outbred Himalayan white-spotted guinea pigs (Klecak et al., 1977) and rabbits (Okabe et al., 1990) was considered moderate and low, respectively. Bagley et al. (1996) found an in vivo study of rabbit skin irritation that ranked *d*-limonene 3.5 out of 8 based on the primary irritation index (PII), and Tsuji et al. (1974) reported that *d*-limonene produced irritation to the eyes of rabbits. Although *d*-limonene was once considered the main allergen in citrus fruits, animal studies showed air-oxidized *d*-limonene, not unoxidized limonene, as the sensitizing agent. Klecak et al. (1977) tested limonene (unspecified form and unknown purity of the test material) in four different sensitization assays with outbred Himalayan white-spotted guinea pigs (the open epicutaneous test, the maximization test, Draize test, and a test with Freund's complete adjuvant [FCA]) and found that *d*-limonene was sensitizing in all but the Draize test. Maisey and Miller (1986) found no sensitization for *d*-limonene in female BALB/*c* mice. Karlberg et al. (1991) tested hydroperoxides and other oxidation products of *d*-limonene with FCA for Dunkin–Hartley albino guinea pigs and reported that these agents were potent contact allergens, whereas unoxidized *d*-limonene induced no marked sensitization.

Developmental and Reproductive Toxicity

There is no apparent evidence that limonene produces teratogenic or embryotoxic effects (Table 5) in the absence of maternal toxicity (WHO, 1998). Tsuji et al. (1975a) examined Wistar rats orally administered d-limonene (0, 591, or 2,869 mg/kg/d) on d 9–15 of gestation and found decreases in body weight, increased number of deaths among dams, and delayed ossification, as well as reduction in total body and organ weight such as thymus, spleen, and ovaries in the offspring. Kodama et al. (1977a) administered orally *d*-limonene (0, 591, or 2363 mg/kg/d) to ICR mice on d 7–12 of gestation, which resulted in reduced growth in dams and a significantly increased incidence of skeletal anomalies and delayed ossification in the offspring. In another study by Kodama et al. (1977b), oral administration of *d*-limonene (250, 500, or 1000 mg/kg/d) to Japanese white rabbits on d 6–18 of gestation showed no dose-related effects in the offspring. At the highest dose, there were some deaths accompanied by reduction in weight gain among dams, and at the intermediate dose growth retardation was noted.

REGULATORY STATUS

Using *d*-limonene for cosmetics is restricted in Korea and the European Union (EU), which have the same specific requirements where the peroxide value of limonene needs to be less than 20 mmol/L (EC, 2010; KFDA, 2010). In addition, the EU Cosmetics Directive 76/768/EEC states that the presence of limonene must be included in the list of ingredients if its concentration exceeds 0.001% in "leave-on" and 0.01% in "rinse-off" products (EC, 2010). On the other hand, there are no regulations for the use of *d*-limonene as an ingredient in cosmetics in the United States, Canada, and Japan (Health Canada, 2011; JMHLW, 2000; U.S. FDA, 2012) (Table 6).

RISK ASSESSMENT

Hazard Identification

The specific nephropathy observed in mature male rats does not appear to be relevant to humans because (1) the type of protein that binds to *d*-limonene or *d*-limonene-1,2-oxide does not function equally in humans and (2) evidence of genotoxic

TABLE 5. Teratogenic, Developmental, and Reproductive Toxicity

Studies	Species	Route	Time	Dose	Results	Reference
Reproductive toxicity	Rat (Wistar)	Oral	Gestation d 9–15	0, 591, or 2869 mg/kg/d	Mother: decrease in body weight and death Offspring: delayed ossification and decrease in total body and organ weights	Tsuji et al. (1975a)
	Mouse (ICR)	Oral	Gestation d 7–12	0, 591, or 2363 mg/kg/d	Mother: reduced growth Offspring: an increased incidence of skeletal anomalies and delayed ossification	Kodama et al. (1977a)
	Rabbit (Japanese white)	Oral	Gestation d 6–18	250, 500, or 1000 mg/kg/d	Mother: some deaths and decreases in weight gain for the highest dose group Offspring: no dose-related effects	Kodama et al. (1977b)
Developmental toxicity	—	—	—	—	No evidence	WHO (1998)
Teratogenicity	—	—	—	—	No evidence	WHO (1998)

TABLE 6. Regulatory Status

			Restrictions		
Country	Agency	Substance	Maximum authorized concentration	Other limitations	Reference
Korea	Korea Food and Drug Administration	D-limonene	Restricted	Peroxide value less than 20 mmol/L	Korea FDA (2010)
		dl-Limonene	Restricted	Peroxide value less than 20 mmol/L	
		l-Limonene	Restricted	Peroxide value less than 20 mmol/L	
United States	U.S. Food and Drug Administration			n/a	U.S. FDA (2000)
European Union	European Commission	*d*-Limonene	Restricted	Peroxide value less than 20 mmol/L	European Commission (2011)
		dl-Limonene (racemic)	Restricted	Peroxide value less than 20 mmol/L	
		l-Limonene	Restricted	Peroxide value less than 20 mmol/L	
Japan	JMHLW		n/a		JMHLW (2000)
Germany		*d*-Limonene		110 mg/m^3	NIOSH (2005)
Sweden		*d*-Limonene		150 mg/m^3	IARC (1999)
Canada	Health Canada		n/a		Health Canada (2011)

Note. KFDA, Korea Food & Drug Administration: JMHLW, Japan Ministry of Health, Labor, and Welfare. mg/m^3 = ppm × molecular weight (g)/24.45.

effects of both *d*-limonene and *d*-limonene-1,2-oxide is absent (Dietrich and Swenberg, 1991; Flamm and Lehman-McKeeman, 1991). The protein content of human urine is different from rat. Specifically, humans excrete little protein (if any), whereas male rats excrete up to 1% (Olson et al., 1990). In addition, there is no α_{2u}-globulin in human plasma or urine, and no α_{2u}-globulin-like protein has been detected in human kidney tissue (Borghoff and Lagarde, 1993). Although *d*-limonene-1,2-oxide binds to α_{2u}-globulin, no other proteins, particularly those synthesized by humans, bind to *d*-limonene-1,2-oxide (Lehman-McKeeman and Caudill, 1992). Similarly, α_{2u}-globulin may play an important role in the development of neoplastic and nonneoplastic kidney lesions, but the mode of its action is not considered

relevant to human risk assessment (Whysner and Williams, 1996).

After oral or ip administration of limonene, another target organ in animals (except for male rats) is the liver (Ariyoshi et al., 1975; NTP, 1990). Exposure to limonene affects organ weight, enzymes, and the bile flow in the liver (Table 2).

Effects on Humans

Falk–Filipsson et al. (1993) reported that all 8 subjects showed no discomfort, irritation, or symptoms related to the central nervous system during their 2-h inhalation exposure to *d*-limonene at 10, 225, or 450 mg/m^3, but there were quantitative decreases in vital capacity following exposure to the highest concentration.

York et al. (1995) examined 4 patch-testing systems using volunteers and found that perfume-grade *d*-limonene produced strong reactions in all types of patches within 10–15 min of exposure. Skin irritation was also tested before the application as well as immediately and 1, 24, 48, and 72 h after removal of the patch. Upon removal of the patch, sensory effects and urticarial responses were evident. In addition, there was a significant irritation for 24 h, and these reactions persisted for 48 and 72 h in many volunteers (York et al., 1995). In a subject dermally treated with *d*-limonene (98%) for 2 h, burning, itching, and a long-lasting purpuric rash were observed (Falk et al., 1991).

Igimi et al. (1976, 1991) examined human volunteers to dissolve gallstones by infusing limonene directly into the bile system, and pain in the upper abdomen, nausea, vomiting, and diarrhea were reported associated with increases in serum aminotransferase and alkaline phosphatase. In addition, the single oral administration of 20 g limonene to 5 healthy male adult volunteers resulted in diarrhea, painful constrictions, and proteinuria but no significant changes such as serum aminotransferase and alkaline phosphatase (Igimi et al., 1976). Rycroft (1980) found that dipentene (dl-limonene), a racemic mixture form of limonene, induced contact dermatitis, while Cachao et al. (1986) found that 15 out of 22 subjects allergic to turpentine oil also reacted to dipentene. Karlberg et al. (1991) conducted patch tests for dermatitis patients from Sweden and Belgium and showed positive reactions in 1.5–2% of subjects tested with oxidized *d*-limonene, which is consistent with observations for other common sensitizers such as formaldehyde.

In a study of 2273 patients at 4 dermatologic clinics in Europe, Matura et al. (2003) demonstrated that approximately 60% of R-(+)-limonene-allergic patients showed positive reactions in patch testing, suggesting oxidized limonene is responsible for its sensitization effect. Although the sensitization potential of oxidized R-(+)-limonene was previously reported by Matura et al. (2002), oxidized S-(+)-limonene was also suggested to act as a sensitizing agent (Matura et al., 2006). However, Greif (1967) found no sensitizing effect in 25 volunteers exposed to limonene in a human maximization test.

Effects on Animals

In the report NTP (1990), a dose-dependent nephropathy in the kidney of male rats was observed after oral administration of *d*-limonene. This kidney lesion, which consisted 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 the hyaline droplet (α_{2u}-globulin accumulation) nephropathy associated with the accumulation of α_{2u}-globulin in tubular cells (Alden, 1986). Some compounds, including decalin—an alicyclic hydrocarbon, Stoddard solvent—a mixture of straight and branched-chain paraffins, and naphthenes and alkyl aromatic hydrocarbons act by structurally fitting into the hydrophobic pocket of α_{2u}-globulin. When the hydrogen bonding between *d*-limonene and α_{2u}-globulin occurs, the digestion of α_{2u}-globulin by lysosomal proteases is inhibited, resulting in limonene accumulation in lysosomes of the P2 segment cells of renal proximal tubules (Lehman-McKeeman et al., 1990; Hard and Whysner, 1994). Molecular modeling studies demonstrated that limonene

exerts a potent structure–activity relationship (SAR) with respect to α_{2u}-globulin binding (Borghoff et al., 1991). This accumulation of α_{2u}-globulin is cytotoxic and leads to single-cell necrosis (Dietrich and Swenberg, 1991). The exfoliated renal epithelium is restored by compensatory cell proliferation, and renal cell proliferation associated with α_{2u}-globulin is reversible. This type of nephrotoxicity has not been observed in immature rats producing no α_{2u}-globulin, and other mammals such as mice, dogs, monkeys, and humans (Alden, 1986; Dietrich and Swenberg, 1991; Kanerva et al., 1987; NTP, 1990; Webb et al., 1989, 1990). The nephropathogenesis and development of renal cancer by such compounds indicate that it is a process specific to mature male rats producing α_{2u}-globulin for nongenotoxic chemicals. Acute and chronic renal effects induced in male rats by limonene are thus unlikely to occur in any species producing no α_{2u}-globulin (U.S. EPA, 2004).

DOSE-RESPONSE ASSESSMENT

To calculate a reference dose (RfD) for humans, data from the 2-yr chronic animal studies were analyzed to obtain a NOAEL (NTP, 1990). The NOAEL is considered to be 250 mg/kg/d for male B6C3F1 mice because there was no evidence of carcinogenic activity of *d*-limonene at doses of 250 or 500 mg/kg. In addition, the NOAEL is considered 500 mg/kg/d for female B6C3F1 mice because there was no evidence of carcinogenic activity of *d*-limonene at doses of 500 or 1000 mg/kg. The NOAEL for male rats may not be relevant for humans because of the species-specific adverse effect, and therefore the lowest NOAEL of 250mg/kg/d for male B6C3F1 mice was selected to calculate the RfD:

$$\mathrm{RfD} = \frac{\mathrm{NOAEL}}{\mathrm{UF_A} \times \mathrm{UF_H}}$$

$$= \frac{250\mathrm{mg/kg/day}}{10 \times 10}$$

$$= 2.5\,\mathrm{mg/kg/day}$$

An uncertainty factor of 10 for intraspecies differences in humans (UF_H) as well as 10 for interspecies differences between animals and humans (UF_A) was used. Thus, from the NOAEL, the RfD calculated for the ingestion of *d*-limonene by humans was 2.5 mg/kg/d.

EXPOSURE ASSESSMENT

The major source of exposure to limonene is food. Limonene occurs naturally in citrus fruits and spices, and is used as a flavor and fragrance additive (U.S. EPA, 1994). However, its intake varies widely across individuals because of diverse dietary patterns. The daily U.S. consumption of *d*-limonene per capita for the general population is estimated to be 0.27 mg/kg/d (FEMA, 1991). A guidance value for ingestion of limonene was calculated to be 0.1 mg/kg/d (WHO, 1998), and the concentration of *d*-limonene ranged from 49 to 2,300 ppm in candy and chewing gum (Sun, 2007). For the general population, dermal exposure to limonene is mainly from contact with household cleaning products in which limonene is used as a fragrance and may be lower than from inhalation exposure in humans (Falk et al., 1991).

RISK CHARACTERIZATION

Based on various data, the systemic exposure dose (SED) and margin of exposure (MOE) were derived as follows:

The average level of exposure to cosmetics for adults (A) in Table 7 was obtained from the Cosmetic, Toiletry, and Fragrance Association (CTFA, 2005). The actual concentration of *d*-limonene in cosmetics (C) was conservatively assumed to be 1% because of lack of data. In addition, the most conservative assumption was used for the value of (D) because there are no recent studies regarding percutaneous absorption ratio. A systemic exposure dose (SED) of 1.48 mg/kg/d was derived from the preceding data.

The MOE was calculated as 169, and therefore estimated exposure to *d*-limonene was

TABLE 7. Calculation of the Margin of Exposure (MOE)

Item	Value	Description	Reference
Average level of exposure to cosmetics for adults (A)	17.76 g	17.76 g of cosmetics/adult/d	CTFA (2005)
Average weight of adults (B)	60 kg	60 kg/adult	
Concentration of *d*-limonene in cosmetics (C)	1%	Assumed value	
Absorption ratio via the percutaneous route (D)	50%	1 g of absorbed *d*-limonene/g of *d*-limonene	
Systemic exposure dose (E)	1.48 mg/kg/d	(A)/(B) × [(C) × (D)]	
NOAEL (F)	250 mg/kg/d	Increased hepatocyte nuclei and cytomegaly in male mice	NTP (1990)
Margin of exposure (MOE)	169	(F)/(E)	

considered to be marginally safe, considering conservative estimation (average use and 100% absorption). Based on the SED, the hazard index (HI) was determined as follows:

$$\mathrm{HI} = \frac{\mathrm{SED}}{\mathrm{RfD}}$$

$$= 1.48\,(\mathrm{mg/kg/d})/2.5\,(\mathrm{mg/kg/d}) = 0.59$$

The calculated HI for *d*-limonene is less than 1, which is acceptable for the criteria of human safety.

SUMMARY AND CONCLUSIONS

Humans are exposed to limonene via food, air, pharmaceuticals, and consumer products because of its wide use as a fragrance. To evaluate the safety of limonene, toxicological studies were conducted both in vitro and in vivo. Repeated-dose toxicity studies showed that *d*-limonene possesses low toxicity in animals when administered orally. Kidney tumors were observed for specifically in mature male rats, but mode of action is regarded as not relevant to humans (Dietrich and Swenberg, 1991; Webb et al., 1989; U.S. EPA, 2004). Limonene produced skin irritation in experimental animals and humans and eye irritation in rabbits (Falk et al., 1991; Klecak et al., 1977; Okabe et al., 1990; York et al., 1995). Contact dermatitis was induced in guinea pigs only by air-oxidized *d*-limonene (Karlberg et al., 1991). In addition, reproductive toxicity was observed but only at exceedingly high doses of *d*-limonene (Tsuji et al., 1975a; Kodama et al., 1977a). Other than the adverse effects described in the preceding, no apparent toxic effects of *d*-limonene, including genotoxicity and teratogenicity, were reported. Thus, limonene is categorized as a chemical with low toxicity except for an irritation and sensitizing property.

The liver has been identified as another critical organ following oral administration of *d*-limonene. The risk characterization of limonene demonstrated that an MOE of 169 and an HI of 0.59 are within the acceptable criteria for human safety (100 and 1, respectively). With the application of the daily U.S. *d*-limonene consumption of 0.27 mg/kg/d, an HI of 0.108 was determined, which is approximately 10-fold lower than an RfD value of 2.5 mg/kg/d. With conservative estimation taken into consideration, *d*-limonene appears to exert no serious risk to human health.

Based on safety evaluations and risk assessments, use of *d*-limonene in cosmetics and consumer products may be considered as safe under the current regulation on cosmetics. However, a comprehensive dermal irritation study of metabolites or oxidation products of *d*-limonene is suggested because the no-expected-sensitization-induction level (NESIL) for skin irritation or sensitization may be needed to establish the acceptable exposure level (AEL) for cosmetics and consumer products separately.

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