Limonene (d-)

Botanical Source

Synonyms MENTHADIENE (para-1,8(9)-),MENTHA-1,8-DIENE (d-para-),CARVENE ([+]-),LIMONENE ([+]-),CITRENE,LIMONENE,LIMONENE [R]-form

IUPAC Name

CAS Reference 5989-27-5

E Number

Food Legislation

Council of Europe (CoE)

Number	Comment
491	COE4

US Food and Drug Administration

Number	Comment
Y;182.60	

Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Number	ADI	Comment
1326	Not Specified	Use of d-limonene as a flavouring agent is subsumed in the 1993 ADI 'not specified', which was maintained at the sixty-third meeting.

FEMA

FEMA No.	Comment
2633	

Natural Occurrence and Use in Food

LIMONENE(d) - FEMA AND FDA; Conforms to the tobacco additive laws in the UK, France and Germany; found in a variety of foods, in particular the citrus fruits, such as lime and orange. It is used in beverages, chewing gum, ice cream, baked goods, confectionery and puddings.

Estimated Intake From Food and Drink

Daily Intake mg/kg/day	FEMA Possible Average Daily
400	115.657

Tobacco Legislation

Tobacco				
Country	Cigarettes	RYO	Cigars	Pipe
Afghanistan				
Algeria				
Argentina				
Australia				
Bahrain				
Brazil				
Burundi				
Canada				
Comoros				
Djibouti				
EEC				
Egypt				
Eritrea				
EU TPD2				
Fiji				
France	Y	Y	Y	Y
GCC (Bahrain,				
Germany	Y	Y	Y	Y
Hong Kong				
Hungary	Y	Y	Y	Y
Iceland				
Iran				
Iraq				
Jordan				
Kazakhstan				
Kuwait				
Latvia				
Libya				
Macedonia				
Madagascar				

Limonene (d-)

Malaysia				
Maldives				
Mexico				
Moldova				
Montenegro				
New Zealand				
Nigeria				
Norway				
Pakistan				
Palestine				
Papua New Guinea				
Rwanda				
Samoa				
Saudi Arabia				
Serbia				
Solomon Island				
Somalia				
Somaliland				
South Sudan				
Sri Lanka				
Sweden				
Switzerland	Y	Y	Y	Y
Syria				
Tunisia				
Turkey				
UK	0.0001	0.0001		0.001
United Arab				
Uzbekistan				
Vietnam				

Y=Permitted for use in tobacco products. If use is limited, the maximum permitted level is given.

Tobacco Product related Chemical and Biological Studies

Smoke Chemistry		
Published Source	Level Tested %	Comment

Ames Activity		
Published Source	Level Tested %	Comment

Micronucleus		
Published Source	Level Tested %	Comment

Neutral red		
Published Source	Level Tested %	Comment

Inhalation		
Published Source	Level Tested %	Comment

Mouse Skin Painting		
Published Source	Level Tested %	Comment

Toxicological Data on the Unburnt Ingredient

[+ve positive; -ve, negative; ? equivocal

With, with metabolic activation; without, without metabolic activation]

In vitro Studies

Species	Test Conditions	End Point	Results	Reference
Mouse lymphoma L5178Y cells	Gene mutation at the *tk* locus with up to 0.06 mg/plate *d*-limonene tested with and without metabolic activation	Mutation	-ve	Nielsen et al, 2013
Chinese hamster ovary cells	Sister chromosome exchange assay in which up to 0.162 mg/plate *d*-limonene was tested with and without metabolic activation	Chromosome effects	-ve	Nielsen et al, 2013
Chinese hamster ovary cells	Chromosome aberration assay in which up to 0.5 mg/plate *d*-limonene was tested with and without metabolic activation	Chromosome damage	-ve	Nielsen et al, 2013
Salmonella typhimurium, strains TA100, TA98, TA1535, TA1537, TA1538.	Bacterial reverse mutation (Ames) test with and without metabolic activation dosing up to 2.72 mg/plate	Mutation	-ve	Nielsen et al, 2013
Salmonella typhimurium, strains TA100, TA98, TA1535, TA1537.	Bacterial reverse mutation (Ames) test with and without metabolic activation dosing up to 3.33 mg/plate	Mutation	-ve	Nielsen et al, 2013

In vivo Studies

Species	Test Conditions	End Point	Results	Reference

Human	The hand of an individual was exposed to *d*-limonene for 2 hours (98%)	Skin irritation.	A burning sensation and painful itching was reported within a few minutes of exposure, the sensation increased in intensity during exposure, the burning sensation persisted for some 10 minutes after exposure. Skin was found to be swollen and reddened. Swelling subsided 90 minutes following exposure. 4.5 hours after exposure small bleeding sites appeared on the skin, persisting for 1-2 weeks.	Nielsen et al, 2013
Rabbit	*d*-Limonene was applied under occlusive cover to intact or abraded rabbit skin for 24 hours @ 100%	Skin irritation	Moderately irritating	Nielsen et al, 2013
Rabbit	*d*-Limonene 25, 40 or 100% in ethanol was applied to the ears of 25 rabbits.	Skin irritation	There was no long term irritation noted on the ears of the rabbits exposed to the lower concentrations. Undiluted *d*-limonene applied to the ears caused skin redness	Nielsen et al, 2013
Rabbit	*d*-limonene was applied to the eyes	Eye irritation	Irritation in the eye was reported.	Nielsen et al, 2013
Human	179 patients with contact allergy to perfume were patch tested with *d*-limonene	Skin sensitization	2 positive reactions [1.1%] were reported.	Nielsen et al, 2013
Human	A review of causes of allergic contact eczema.	Skin sensitization	[No] reporting of *d*-limonene as the single cause for allergic contact eczema	Nielsen et al, 2013
Mouse	Skin sensitization study in mice	Skin sensitization.	Not sensitizing	Nielsen et al, 2013
Guinea pig	Unoxidised *d*-limonene was tested with Freund's complete adjuvant.	Skin sensitization	Not sensitizing	Nielsen et al, 2013
Mouse, CBA/J	Local lymph node assay. Groups of 4 females were administered 25 µl *R-(+)*-limonene in acetone olive oil (4:1), topically for 3 days. On day 5, mice received [^{3}H]-methyl thymidine intravenously and, 5 hours later, mice were sacrificed and lymph nodes excised and analysed. Dose: 0, 1, 25 or 100%.	Skin sensitization	Weak sensitizer. [OECD test guideline 429 defines stimulation index (SI) as the ratio of lymphocyte proliferation in a treated group to that in the vehicle control group. An SI of ≥3 is considered positive and the estimated concentration three (EC3) is the estimated concentration of a test substance needed to produce an SI of 3. An EC3 >10 is indicative of weak sensitizing potential.]	Wei et al, 2010
Mouse, BALB/c.	6 male mice (per group) were exposed nose only to 75 mg/m^3 D-limonene on three consecutive days for	Respiratory sensitization	No sensitization	ter Burg et al, 2014

	45, 90, 180 or 360 minutes. Mice were killed 3 days after the last exposure to enable examination of the lymph nodes.			
Human	8 healthy men were exposed to an atmosphere containing 10, 225, 450 mg/m^3 *d*-limonene for 2 hours	Lung function, reported symptoms	No symptoms were reported, a statistically significant 2% decrease in vital capacity was observed at the highest exposure level was not considered clinically or functionally significant by the investigators	Nielsen et al, 2013
Mouse, BALB/c.	Mice were exposed to airborne *d*-limonene for 30 minutes. Up to 1600 ppm [8900 mg/m^3]	Local effects – Respiratory irritation and General systemic effects – Single exposure: Respiratory rate, reflex, observations	RD_{50} 6090 mg/m^3. [the RD_{50} is the exposure concentration associated with a 50% decrease in respiratory rate]. "The effects were reported to be caused by decreased trigeminal reflex due to sensory irritation." No pulmonary or anaesthetic effects were reported.	Nielsen et al, 2013
Human	5 healthy male volunteers were given a single oral dose of 20,000 mg [285 mg/kg bw for a 70-kg individual] *d*-limonene	Reported symptoms, functional tests of the liver, kidney and pancreas.	Functional tests were normal. Proteinuria [protein in the urine], non-bloody diarrhoea, and tenesmus [sensation of incomplete defecation] were reported in all subjects	Nielsen et al, 2013
Rat, Wistar	Groups of 4 male rats were administered *d*-limonene in 2% tragacanth solution by oral gavage. Dose: 0, 200, 400, 600, 800 or 1200 mg/kg bw	Mortality, liver triglycerides, microsomal proteins, enzyme levels.	No effects were found	Nielsen et al, 2013
Mouse	Male and females were dosed orally.	Mortality	LD_{50} 5600 mg/kg bw (male). LD_{50} 6600 mg/kg bw (female).	Nielsen et al, 2013
Rat	Male and females were dosed orally	Mortality	LD_{50} 4400 mg/kg bw (male). LD_{50} 5100 mg/kg bw (female).	Nielsen et al, 2013
Rat	Single oral administration	Mortality	LD_{50} >5000 mg/kg bw	Nielsen et al, 2013
Rabbit	Single dermal administration	Mortality	LD_{50} >5000 mg/kg bw	Nielsen et al, 2013
Rat, Fischer 344.	Groups of 5 male rats were administered 75, 150, 300 mg/kg bw *d*-limonene by oral gavage, 5 days/week for 6 or 27 days	Liver and kidney weight, histological examination of the kidneys.	Relative liver and kidney weights were increased in a dose related manner. Hyaline [protein] droplets and increased levels of globulin proteins were observed in the kidneys. Chronic nephrosis [degeneration of kidney tubules] was observed in rats used killed at the later timepoint.	Nielsen et al, 2013
Rat	Groups of 5 male and females were exposed orally to 0, 277, 554,	Body weight, food intake, organ weights,	Granular casts were seen in the kidneys of most exposed males in all treated groups.	Nielsen et al, 2013

	1385 or 2770 mg/kg bw/day *d*-limonene for 30 days	urinalysis, haematology, biochemical analysis, histopathology.	No other effects were reported	
Rat, F344/N	Groups of 5 rats (per sex, per dose) were administered 0, 413, 825, 1650, 3300 or 6600 mg/kg bw/day *d*-limonene in corn oil by oral gavage, 5 days per week for 16 days (12 doses)	Mortality, clinical signs of toxicity, histopathology.	Mortality was 10/10 at 6600 and 8/10 at 3300 mg/kg bw/day. No clinical signs were reported in rats receiving 1650 mg/kg bw/day or less. No histopathological effects were reported	Nielsen et al, 2013
Rat, NCI Black Reiter.	Male rats were administered 1650 mg/kg bw/day *d*-limonene by oral gavage daily for 4 days	Examination of the kidney	No nephrotoxicity was found. [NCI Black Reiter rats do not synthesize the alpha$_{2u}$-globulin present in the hyaline droplets found in the kidneys of other treated strains.]	Nielsen et al, 2013
Rat, Wistar	Male rats were administered 0 or 400 mg/kg bw/day *d*-limonene in 2% tragacanth by oral gavage for 2, 3, 15 or 30 days	Liver weight, hepatic phospholipid content, cholesterol levels, liver lipid and enzyme levels.	Relative liver weight and liver phospholipid content was slightly increased in rats receiving treatment for 30 days. Alterations in liver lipid and enzyme levels were found	Nielsen et al, 2013
Rat, Fischer 344; NBR	0 or 150 mg/kg bw/day *d*-limonene was administered in corn oil to groups of male rats by oral gavage for 4 or 31 weeks	Examination of the kidney	Cell proliferation in the kidneys was noted in Fischer 344 but not NBR rats. [NCI Black Reiter rats do not synthesize the alpha$_{2u}$-globulin present in the hyaline droplets found in the kidneys of other treated strains.]	Nielsen et al, 2013
Rat, Fischer 344	0, 5, 30, 75 or 150 mg/kg bw/day *d*-limonene was administered orally to groups of rats for 91 days	Examination of the kidney	Hyaline droplets were noted in the kidney and increased proliferating cell nuclear antigen-labelled renal proximal tubular cells at 30 mg/kg bw/day and above, but not at 5 mg/kg bw/day.	Nielsen et al, 2013
Mouse, C57BL/6-derived transgenic mice engineered to express alpha$_{2u}$-globulin	Mice were administered 0 or 150 mg/kg bw/day *d*-limonene for 3 days.	Examination of the kidneys	Spontaneous hyaline droplet formation was not seen in control animals but small droplets were seen in animals that had received *d*-limonene.	Nielsen et al, 2013
Dog, Beagle	Male and female dogs were administered *d*-limonene daily, for 6 months. Dose Including 340 and 1000 mg/kg bw/day (females). Including 1000 and 3024 mg/kg bw/day (males).	Bodyweight, clinical signs of toxicity	Protein casts in the renal tubules were seen in females at 340 mg/kg bw/day and above and in males at 1000 mg/kg bw/day and above. Slight weight loss and frequent vomiting in some animals was reported in females at 1000 mg/kg bw/day and above and in males at 3024 mg/kg bw/day and above.	Nielsen et al, 2013
Dog, Beagle	100 or 1000 mg/kg bw.*d*-limonene was	Kidney weight, histopathological	Kidney weights were increased; there were no	Nielsen et al, 2013

	administered by twice-daily oral gavage to dogs for 6 months; the top dose was limited by emesis.	examination of the kidney.	histopathological changes in the kidney (including an absence of hyaline droplets and nephropathy).	
Rat, Sprague-Dawley.	Single cell gel/comet assay in which 0, 1000 or 2000 mg/kg bw *d*-limonene in 0.5% carboxymethyl cellulose was administered to 4 male rats (per dose) by oral gavage. 3-6 or 22-26 hours later, treated animals were killed and kidney cells analysed. Positive and negative controls were used.	DNA damage	-ve	ECHA, 2014a
Rat, Big blue	Transgenic animal mutagenicity assay. 10 male transgenic rats were fed a diet containing 0 or 1% *d*-limonene for 10 days. After a 14-day period the rats were killed and liver and kidney tissue DNA was isolated and assayed for mutation	DNA damage	-ve	ECHA, 2014a
Rat, Wistar	Comet assay in which 0 or 2000 mg/kg bw *d*-limonene in olive oil was administered to groups of 4 male rats (per dose) by oral gavage. Treated animals were killed 3, 8 and 24 hours after dosing and kidney, bladder, lung, brain and bone marrow cells analysed. Vehicle only and negative controls were used.	DNA damage	-ve	ECHA, 2014a
Mouse, ddY	Comet assay in which 0 or 2000 mg/kg bw *d*-limonene in olive oil was administered to groups of 4 male mice (per dose) by oral gavage. Treated animals were killed 3, 8 and 24 hours after dosing and stomach, colon, kidney, bladder, lung, brain and bone marrow cells analysed. Vehicle only and negative controls were used	DNA damage	-ve	ECHA, 2014a

Mouse	Mammalian spot test. Mice were administered 215 mg/kg bw/day *d*-limonene via 3 intraperitoneal injections	Mutation	-ve	Nielsen et al, 2013
Rat, Fisher 344/N	Groups of 50 rats (per sex, per dose) were administered *d*-limonene in corn oil by oral gavage, 5 days a week for 103 weeks. The experiment was terminated after 105 weeks. Dose: 0, 75, 150 mg/kg bw/day (males). 0, 300 or 600 mg/kg bw (females)	General systemic effects – Repeated exposure and Carcinogenicity: Examination of the kidney.	General systemic effects – Repeated exposure: LOAEL 75 mg/kg bw/day (males) (the lowest dose tested) NOAEL 600 mg/kg bw/day (females) (the highest dose tested). Mineralisation and epithelial hyperplasia were seen at increased frequency in the kidneys of treated males; no such effects were seen in females. Carcinogenicity: Some evidence of carcinogenicity in males, but not in females. A dose-dependent increased incidence of renal tube hyperplasia and renal tubular adenocarcinoma was observed. There were no such kidney lesions in females.	Nielsen et al, 2013
Mouse, $B6C3F_1$	Groups of 50 rats (per sex per dose) were administered *d*-limonene in corn oil by oral gavage, 5 days a week for 103 weeks. The experiment was terminated at 105 weeks. Dose: 0, 250 or 500 mg/kg bw (males). 0, 500 or 1000 mg/kg bw (females)	General systemic effects – Repeated exposure: Bodyweight, clinical signs of toxicity, gross necropsy. Carcinogenicity: Gross necropsy.	General systemic effects – Repeated exposure: NOAEL 500 mg/kg bw/day (the highest dose tested in males) Mean body weights were reduced in top dose females but no other toxic effects were reported. Carcinogenicity: No evidence of carcinogenicity. No significant increase in the incidence of neoplasm was observed	Nielsen et al, 2013
Rat, Wistar	Pregnant rats were administered 0, 591 or 2869 mg/kg bw/day *d*-limonene orally from day 9 to 15 of gestation	Developmental and maternal toxicity: bodyweight, foetal mortality, skeletal examination of the foetuses, organ weights	NOAEL 591 mg/kg bw [maternal and developmental]. Maternal bodyweight gain was decreased and "several" dams died in the top dose group and foetal deaths were also increased. "Significantly delayed ossification" [formation of bone] of the metacarpal bone and proximal phalanx was seen in the high dose group foetuses together with decreased total bodyweight,	Nielsen et al, 2013

			and decreased thymus, spleen and ovary weights	
Mouse, ICR	Pregnant mice received 0, 591 or 2363 mg/kg bw/day *d*-limonene from days 7 to 12 of gestation.	Developmental and maternal toxicity: bodyweight, foetal gross necropsy	NOAEL 591 mg/kg bw/day [maternal and developmental]. "Significant decreases" in bodyweight gain of dams at 2363 mg/kg bw/day. In the foetuses in this group, delayed ossification [bone formation] and skeletal abnormalities were found as well as decreased bodyweight gain	Nielsen et al, 2013
Rabbit, Japanese white.	Rabbits were administered 0, 250, 500 or 1000 mg/kg bw/day *d*-limonene from day 6 to day 18 of gestation	Developmental and maternal toxicity: bodyweight, mortality, examination of the foetuses	"Significant decreases in bodyweight" were seen in dams that received the two lower doses and 33% mortality was reported in top dose females. The investigators considered that *d*-limonene was not teratogenic; some lung defects and delayed ossification was seen but was not statistically significant when compared with controls. According to Nelsen et al, 2013, another reviewer (Nord, 1993) considered 250 mg/kg bw to be an NOAEL for maternal and developmental toxicity.	Nielsen et al, 2013

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