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Washington D.C. 20006
Tel. (202)-331-2325 Fax (202)-463-8998

May 21, 2002

Christie Todd Whitman, Administrator
US EPA
P.O. Box 1473
Merrifield, VA 22116
Attn: Chemical Right-to-Know Program

Dear Ms. Whitman:

On behalf or the member companies of the Terpene Consortium, the Flavor andFragrance High Production Volume Consortia is pleased to submit the Test Plan andRobust Summaries for the chemical category designated the "Monoterpene Hydrocarbons" to the HPV Challenge Program, AR-201. The Terpene Consortium has chosen not to belong to the HPV Tracker System for submission of test plans and robust summaries. We are therefore submitting the test plan and accompanying robust summaries directly to EPA to make available to the public. This submission includes one electronic copy in pdf. format. A hard copy of this submission is available upon request. The EPA registration number for the Terpene Consortium is

Please feel free to contact me with any questions or comments you might have concerning the submission at tadams@therobertsgroup.net, tadams@chemintox.com or 202-331-2325.

Sincerely,
Timothy Adams, Ph.D.
Technical Contact Person for FFHPVC

AR201-13756A

The Flavor and Fragrance High Production Volume Consortia

The Terpene Consortium

Test Plan for Monoterpene Hydrocarbons

d-Limonene	CAS No. 5989-27-5
dl-Limonene	CAS No. 138-86-3
Terpinolene	CAS No. 586-62-9
Myrcene	CAS No. 123-35-3
Dihydromyrcene	CAS No. 2436-90-0
Hydrocarbons, terpene processing by-products	CAS No. 68956-56-9
Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	CAS No. 8008-57-9
Terpenes & terpenoids, sweet orange oil	CAS No. 68647-72-3
Terpenes & terpenoids, turpentine oil, limonene fraction	CAS No. 65996-99-8
Terpenes & terpenoids, limonene fraction	CAS No. 65996-98-7
Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue	CAS No. 68334-40-7
Terpenes & terpenoids, turpentine-oil residue	CAS No. 68938-00-1

FFHPVC Terpene Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

The Flavor and Fragrance High Production Volume Chemical Consortia

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List of Member Companies

ARIZONA CHEMICAL

BASF CORPORATION

BEDOUKIAN RESEARCH, INC.

BOISE CASCADE CORPORATION

CHAMPION INTERNATIONAL CORPORATION

CITRUS & ALLIED ESSENCES, LTD.

DRAGOCO

FRAGRANCE RESOURCES, INC.

GIVAUDAN FRAGRANCES CORPORATION

HERCULES INCORPORATED

INTERNATIONAL FLAVORS & FRAGRANCES INC.

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KURARAY CO., LTD.

MEAD CORPORATION

MILLENNIUM CHEMICALS

POLAROME INTERNATIONAL INCORPORATED

QUEST INTERNATIONAL

SENSIENT FLAVORS

TECNAL CORPORATION

THE PROCTOR AND GAMBLE CO.

UNILEVER-HPC

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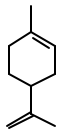
The HPV Challenge Test Plan for Monoterpene Hydrocarbons

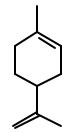
1 Identity of Substances



d-Limonene
$C_{10}H_{16}$

Synonyms:
Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)-
(R)-(+)-p-Mentha-1,8-diene
d-1-Methyl-4-isopropenyl-1-cyclohexene

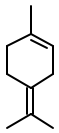
CAS No. 5989-27-5



dl-Limonene
$C_{10}H_{16}$

Synonyms:
Cyclohexene, 1-methyl-4-(1-methylethenyl)-
Dipentene

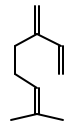
CAS No. 138-86-3



Terpinolene
$C_{10}H_{16}$

Synonyms:
Cyclohexene, 1-methyl-4-(1-methylethylidene)-
p-Mentha-1,4(8)-diene
1-Methyl-4-isopropylidene-1-cyclohexene

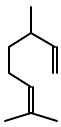
CAS No. 586-62-9



Myrcene
$C_{10}H_{16}$

Synonyms:
1,6-Octadiene, 7-methyl-3-methylene-
7-Methyl-3-methylene-1,6-octadiene
beta-Myrcene

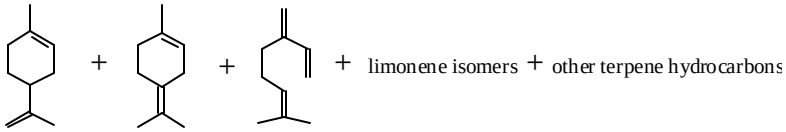
CAS No. 123-35-3



Dihydromyrcene
$C_{10}H_{18}$

Synonyms:
1,6-Octadiene, 3,7-dimethyl-
3,7-Dimethylocta-1,6-diene

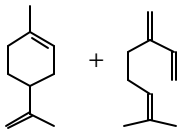
CAS No. 2436-90-0



22-34% 22-33% 5-10% 18% 10%
limonene terpinolene myrcene

Hydrocarbons, terpene processing by-products

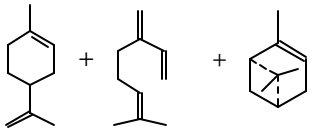
CAS No. 68956-56-9



91-94 % 2.0-2.1%
Limonene *beta*-Myrcene

Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)

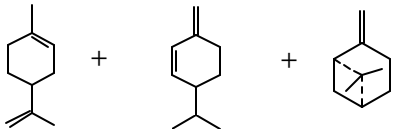
CAS No. 8008-57-9



d-Limonene *beta*-Myrcene *alpha*--Pinene
91-95% 1-3% 1-2%

Terpenes & terpenoids, sweet orange oil

CAS No. 68647-72-3

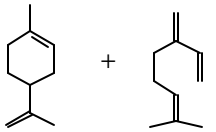


dl-Limonene *beta*-Phellandrene *beta*-Pinene other terpene hydrocarbons

59-64% 14-18% 4-11% 5-10%

Terpenes and terpenoids, turpentine oil, limonene fraction

CAS No. 65996-99-8

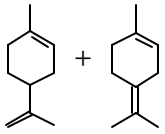


dl-Limonene Myrcene Other terpene hydrocarbons

96-98% 1-2% 5-10%

Terpenes and terpenoids, limonene fraction

CAS No. 65996-98-7

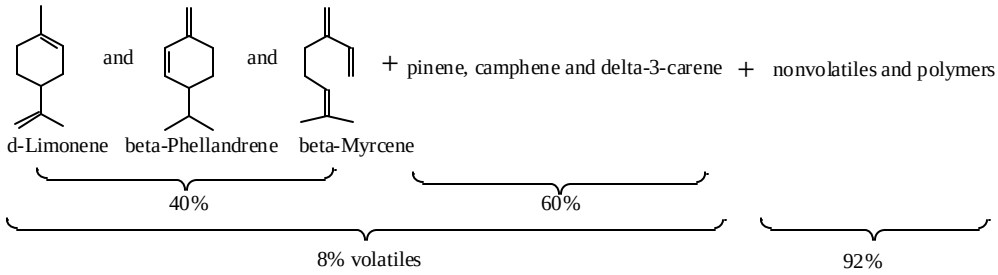


dl-Limonene terpinolene Other diterpene and sequiterpene hydrocarbons Tertiary monoterpene alcohols (mainly linalool and alpha-terpineol) Other oxygenated terpenes

30-35% 1-3% 30-35% 15-20% 5-12%

Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue

CAS No. 68334-40-7



Terpenes & terpenoids, turpentine-oil residue

CAS No. 68938-00-1

2 Category Analysis

2.1 Introduction

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Terpene Consortium, as a member of FFHPVC serves as an industry consortium to coordinate testing activities for terpenoid substances under the Chemical Right-to-Know Program. Twenty-one (21) companies are current members of The Terpene Consortium. The Terpene Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing. The category analysis, test plan, and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

2.2 Background Information

The chemical category designated "Monoterpene Hydrocarbons" includes five simple monoterpene hydrocarbons and seven mixtures comprised primarily of the five terpene hydrocarbons. In plants, monoterpene hydrocarbons are produced by the isoprene pathway. Monoterpene hydrocarbons have a chemical formula of $C_{10}H_{16}$, or if partly or completely saturated, $C_{10}H_{18}$ or $C_{10}H_{20.}$ Monoterpene hydrocarbons are ubiquitous in food [CIVO-TNO, 1999] given that they are present in varying degrees in all plants. Being volatile constituents of plants, they are also normal components of the atmosphere.

Monoterpene hydrocarbons are mainly released by coniferous woodland such as pine trees, cedars, redwood and firs. To a lesser extent, they are also produced and released by deciduous

plants. They are common components of traditional foods occurring in essentially all fruits and vegetables [CIVO-TNO, 1999]. *d*-Limonene, *beta*-myrcene, and terpinolene are currently recognized by the U.S. Food and Drug Administration (FDA) as GRAS ("generally regarded as safe") for their intended use as flavoring substances [Hall and Oser, 1965]. Quantitative natural occurrence data indicate that oral intake of these substances occurs predominantly from consumption of food in which they occur naturally [Stofberg and Grundschober, 1987; Stofberg and Kirschman, 1985]. Greater than 2,500,000 pounds (lbs) of *d*-limonene, 50,000 lbs of terpinolene, and 150,000 lbs of *beta*-myrcene are consumed annually as natural components of food in the United States. The estimated poundage of *d*-limonene, terpinolene and *beta*-myrcene used as flavoring substances in 1995 were 213,000 lbs, 1,170 lbs, 2,620 lbs, respectively [Lucas *et al.*, 1999]. Therefore, greater than 90%, 97% and 98% of intake occurs from consumption of food containing naturally occurring *d*-limonene, terpinolene and *beta*-myrcene, respectively. Based on the annual volume of consumption of *d*-limonene, terpinolene and *beta*-myrcene, it is estimated that the combined average daily *per capita* intake is approximately 1.3 mg/day. Intakes as high as 13 mg/day (eaters only) may be expected for consumers of diets rich in fruits, vegetables, and spices [Oser and Hall, 1977].

As a volatile C_{10} hydrocarbon, limonene is also a naturally occurring component of the atmosphere. Estimates of atmospheric concentrations of limonene in urban indoor air, rural outdoor air (*Pinus* forest canopy), and occupational environments (*e.g.* sawmill or paper mill worker) have been reported to be approximately 0.6-11.1 microgm/m^3, 0.6-1.1 microgm/m^3, and 1.7-240 microgm/m^3, respectively [IPCS, 1998]. Assuming that a human is exposed daily to an urban atmosphere containing 11 ug/m^3 *d*-limonene and that 65% of the inhaled *d*-limonene is absorbed [Falk-Filipsson *et al.*, 1993], the daily intake from atmospheric exposure would be approximately 0.5 mg/day {11 ug/m^3 x 3 m^3/hr x 24hrs/day x 0.65 (absorption rate) x 10^{-3} ug/mg}. When oral and inhalation exposures are combined, it is estimated that average total daily exposure from food consumption and normal inhalation in an urban environment is in the range of 2 mg. However, for specialized eating groups (90% eaters, *e.g.*, vegetarians), daily intakes may easily exceed 20 mg.

2.3 Structural Classification

The chemical category designated terpenoid hydrocarbons includes three simple C_{10} isomeric monocyclic terpene hydrocarbons (*d*-limonene, *dl*-limonene, and terpinolene) two simple C_{10} acyclic terpene hydrocarbons (*beta*-myrcene and dihydromyrcene) and seven mixtures composed primarily of *d*-limonene, *dl*-limonene (dipentene), terpinolene, myrcene, and *alpha*- and *beta*-pinene (see the FFHPVC Test Plan for the Chemical Category Bicyclic Terpenoid Hydrocarbons). *d*-Limonene and terpinolene are monocyclic monounsaturated terpenes. *d*-Limonene is (R)-1-methyl-4-(1-methylethenyl)-cyclohexene, *dl*-limonene is an equal mixture of (R)- and (S)-1-methyl-4-(1-methylethenyl)-cyclohexene while terpinolene is 1-methyl-4-(1-methylethylidene)-cyclohexene. Myrcene is commonly recognized as *beta*-myrcene, the isomeric form that predominates in nature. *beta*-Myrcene is an acyclic monounsaturated isomer of limonene. The *alpha* isomer, 2-methyl-6-methylene-1,7-octadiene is not found in nature [Merck, 1996] and is of no commercial importance. *beta*-Myrcene is 7-methyl-3-methylene-1,6-octadiene while dihydromyrcene is 3,7-dimethyl-1,6-octadiene.

Typical analyses of the seven mixtures in this category reveal that the five chemically defined members of this chemical category are major constituents. Hydrocarbons, terpene processing by-products is primarily composed of limonene, isomers of limonene, terpinolene, myrcene and other terpene hydrocarbons [Arizona Chemical, 1999]. Orange peel oil, sweet (Citrus sinensis (L.) Osbeck) is composed almost completely of *d*-limonene (91-94%) with *beta*-myrcene as a minor constituent (2.0-2.1%). Terpenes and terpenoids, sweet orange oil is primarily *d*-limonene (91-95%), *beta*-myrcene (1-3%), and *alpha*-pinene (1-2%) [Bauer K. and D. Garbe, 1985]. Terpenes and terpenoids, turpentine oil, limonene fraction is primarily racemic (*dl*)-limonene (59-64%), with *beta*-phellandrene (1-methyl-4-isopropyl-1,5-cyclohexadiene,14-18%), *beta*-pinene (4-11%) and other terpene hydrocarbons (5-10%) being minor constituents [Arizona Chemical, 1999]. Terpenes and terpenoids, limonene fraction is composed primarily of racemic limonene (96-98%), myrcene (1-2%) and other terpene hydrocarbons (5-10%) [Arizona Chemical, 1999]. Terpenes and terpenoids, turpentine oil, limonene fraction, distillation residue is composed of limonene (30-35%), other diterpene and

sesquiterpene hydrocarbons (30-35%), tertiary monoterpene alcohols (15-20%), terpinolene (1-3%) and other oxygenated terpenes (5-12%) [Arizona Chemical, 1999]. Terpenes and terpenoids, turpentine-oil residue is composed of polymeric (82%), and nonvolatile terpene (10%) constituents which have no commercial value. This residue is used as fuel at the industrial site at which it is isolated from turpentine. The volatile component (8%) is composed of approximately 40% limonene, phellandrene, and myrcene isomers and 60% pinene, camphene and carene isomers [Arizona Chemical, 2000].

2.4 Industrial and Biogenic Production

2.4.1 Industrial Production

d-Limonene is a liquid with a lemon-like odor and a byproduct from the manufacture of orange juice. It may also be obtained from orange oils through vacuum distillation. It has been estimated that the worldwide production of orange oil (*d*-limonene accounts for greater than 90% of orange oil) is 26,000 tons, with the greatest production occurring in Brazil (17,000 tons) and in the United States (6,900 tons) [Lawrence, 1985]. Limonene is also produced by acid catalyzed isomerization of *alpha*- and *beta*-pinene [Bauer and Garbe, 1985]. Myrcene is largely produced from the pyrolysis of *beta*-pinene at high temperatures [Bauer and Garbe, 1985].

Another industrial source of limonene and *beta*-myrcene is crude sulfate turpentine (CST) obtained as a waste product in the manufacturer of cellulose *via* the sulfate process. Turpentine is derived primarily from *Pinus* species and is used in whole form as a solvent for paints and varnishes or as a cleaning agent. Turpentine is composed of approximately 60-65% *alpha*-pinene, 25-35% *beta*-pinene with the remainder being other terpenoid hydrocarbons including limonene and myrcene. CST obtained from southern paper mills in the United States consists of 4.2% *d*-limonene and 1.7% *beta*-myrcene [Derfer and Traynor, 1992]. It has been estimated that the worldwide production of turpentine is approximately 330,000 metric tons of which almost 100,000 metric tons is gum turpentine and the bulk of the remainder is sulphate turpentine [National Resources Institute, 1995].

Level 1 fugacity calculations using limonene indicate that 93.8% will partition to air at equilibrium. In the atmosphere, limonene rapidly reacts with hydroxyl radicals, ozone and nitrate radicals [NICNAS, 2001]. If it were conservatively assumed that 2% of industrially separated limonene is lost during industrial processing of orange oil and turpentine, the total annual worldwide emission of *d*-limonene from these industrial sources would be approximately 800 metric tons. This can be compared with the biogenic emissions into the air discussed below.

Limonene derived from citrus essential oils and CST, is used as a raw material for the chemical synthesis of a variety of terpene alcohols and ketones such as menthol, carvone and *alpha*-terpineol [Bauer and Garbe, 1985]. Limonene is also used directly but in far less amounts as a fragrance material for household products and as a component in the manufacture of artificial essential oils [Lawrence, 1985].

2.4.2 Biogenic Production

d-Limonene, terpinolene and myrcene naturally occur in many essential oils. Very high levels of *d*-limonene are present in orange oil (greater than 90%), grapefruit oil (90%), lemon oil (70%) and celery oil (60%) etc. [NICNAS, 2001]. *beta*-Myrcene and terpinolene occur naturally in a wide variety of foods including lemon peel oil, orange peel oil, orange juice, and lime juice [CIVO-TNO, 1999].

In a recent study of the measurement of terpene emissions from *Pinus sylvestris* dominated forests [Rinne *et al.*, 2000], it was reported that the main monoterpenes emitted were *alpha*-pinene (57%), *delta*-3-carene (22%), *beta*-pinene/myrcene (14%), limonene (5%) and camphene (3%). The emissions of *d*-limonene and myrcene are not limited to conifers. In a study of emissions over arable crops and a beech forest [Gallagher *et al.*, 2000], all five substances were detected. Indeed landscape flux potentials have been measured in three quite varied sites (an urban forest, a mixed deciduous and coniferous forest, and a mixed shrub oak forest) in the U.S. from each of 63 species of trees [Helmig *et al.*, 1999a, 1999b]. *d*-Limonene, *beta*-myrcene and terpinolene were detected in a substantial proportion of the species measured with fluxes ranging from 0.1 to 67 $\mu gChr^{-1}gdw^{-1}$ (µg carbon per hour per gram dry

weight), 0.1 to 2.6 $\mu gChr^{-1}gdw^{-1}$ and 0.1 to 2.2 $\mu gChr^{-1}$ gdw^{-1}, respectively [Helmig *et al.*, 1999a]. These fluxes have been used to calculate average daily fluxes for each substance at each site [Helmig *et al.*, 1999b]. For *d*-limonene these were 71, 120 and 15 $\mu gCm^{-2}hr^{-1}$ (µg carbon per m^2 per hour), for *beta*-myrcene, 14, 14 and 0.4 $\mu gCm^{-2}hr^{-1}$ and for terpinolene, 5, 15 and 2 $\mu gCm^{-2}hr^{-1}$, as measured above an urban forest, a mixed deciduous and coniferous forest, and a mixed shrub oak forest, respectively. The relative emissions for *d*-limonene is 3.6, 2.7 and 0.6%; for *beta*-myrcene, 0.7, 0.3 and 0.01%; and for terpinolene, 0.3, 0.3 and 0.1% of the total volatile organic compounds (VOC) emissions for each of the three sites, respectively. These figures can be used to estimate the total global emissions of these materials (see below).

In a recent review of natural emissions of volatile compounds [Guenther *et al.*, 2000] it was estimated that in North America the total annual emission of *d*-limonene and myrcene[1] was 0.4 to 1.1 million metric tons; the total annual emission of terpinolene[2] was 0.1 to 0.4 million metric tons. The total global emissions of these three compounds can be estimated in two ways. The total annual global emission of VOCs has been estimated as 1,150 million metric tons [Guenther *et al.*, 1995]. If the same percentage of total emissions of VOCs as has been measured over 3 different forest types, 3.6, 2.7 and 0.6% for *d*-limonene (average = 2.3%), 0.7, 0.3 and 0.01% (0.34%) for *beta*-myrcene and 0.3, 0.3 and 0.1% (0.23%) for terpinolene, it can be estimated that the total annual global emissions for these three substances would be approximately 26.5 million, 4 million and 2.6 million metric tons, respectively. On the other hand, if the average rates of emission of *d*-limonene (69 $\mu gCm^{-2}hr^{-1}$), *beta*-myrcene (9.5 $\mu gCm^{-2}hr^{-1}$) and terpinolene (7.3 $\mu gCm^{-2}hr^{-1}$) are applied to the latest global forest coverage estimates of 3.9 billion hectares [Food and Agriculture Organization, 2000], then annual global biogenic emissions of *d*-limonene, *beta*-myrcene and terpinolene are approximately 23.5 million, 3.2 million and 2.5 million metric tons, respectively.

[1] Emission rate was estimated for d-limonene, sabinene, β-phellandrene, p-cymene and myrcene [Guenther *et al.*, 2000].
[2] Emission rate was estimated for camphene, camphor, bornyl acetate, α-thugene, terpinolene, α-terpinene, γ-terpinene, ocimene, 1,8-cineole, piperitone, α-phellandrene, and tricyclene [Guenther *et al.*, 2000].

Based on the close agreement of estimates of biogenic emissions derived from two separate methods, it is concluded that total annual atmospheric emission of *d*-limonene, *beta*-myrcene and terpinolene is predominantly from biogenic sources. The relative contribution from biogenic and industrial sources can be represented by a global emission ratio (GER = biogenic emission/industrial emission). In the case of *d*-limonene, the GER would exceed 10,000, suggesting that biogenic emissions far exceed anthropogenic emissions. As a result, humans are unavoidably exposed to naturally occurring monterpenoid hydrocarbons including limonene, myrcene and terpinolene.

2.5 Chemical Reactivity and Metabolism

Studies of terpene hydrocarbons indicate that they are rapidly absorbed, distributed, metabolized and excreted. The principal metabolic pathway involves side chain oxidation to yield monocyclic terpene alcohols and carboxylic acids. These metabolites are mainly conjugated with glucuronic acid and excreted in the urine, or to a lesser extent in the feces. A secondary pathway involves epoxidation of either the exocyclic or endocyclic double bond yielding an epoxide that is subsequently detoxicated *via* formation of the corresponding diol or conjugation with glutathione. Although some species- and sex-specific differences exist, studies for *d*-limonene and *beta*-myrcene indicate that the monoterpene hydrocarbons in this chemical category will participate in common pathways of absorption, distribution, metabolism and excretion.

2.5.1 Absorption, Distribution and Excretion

Following oral intake, limonene is completely and rapidly absorbed and distributed throughout the body, preferentially to fatty tissues, as shown by a high oil blood partition coefficient and a long half life during the slow elimination phase [Falk *et al.*, 1990; Falk-Filipsson *et al.*, 1993]. Volunteers exposed *via* inhalation to 450 mg/m^3 *d*-limonene showed three phases of elimination in the blood, with half-lives of about 3, 33, and 750 minutes, respectively [Falk-Filipsson *et al.*,

1993]. Radioactivity in the liver, kidney and blood were negligible 48 hours after oral administration [^{14}C]-*d*-limonene to rats. About 60% of the administered radioactivity was recovered from the urine, with 5% from feces and 2% from expired CO_2 [Igimi *et al.*, 1974]. In a separate study using male human volunteers, 50-80% of an oral dose of ^{14}C-*d*-limonene was excreted in the urine with less than 10% appearing in the feces [Kodama *et al.*, 1976].

2.5.2 Biotransformations

Limonene is metabolized *via* cytochrome P450 to produce polar metabolites, which are conjugated and excreted in the urine. The major metabolic pathway involves oxidation of the 1-methyl group yielding perillic acid [Kodama *et al.*, 1976] (see Figure 1). Limonene is also epoxidized at the 8,9-double bond. The resulting epoxide is hydrolyzed to the corresponding diol by epoxide hydrolase (EH) [Watabe *et al.*, 1981]. Other studies have also indicated minor pathways involving ring hydroxylation and epoxidation of the 1,2-double bond [Kodama *et al.*, 1976; Poon *et al.*, 1996].

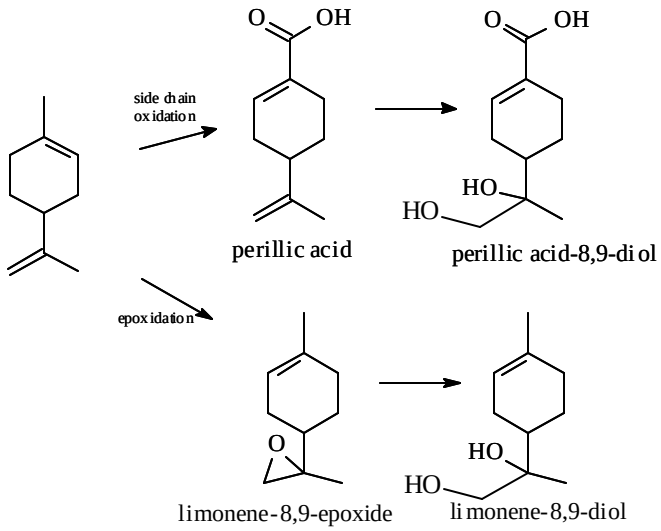
2.5.3 Humans

Limonene given orally to humans yields the following major plasma metabolites: perillic acid, limonene-1,2-diol, limonene-8,9-diol, and dihydroperillic acid, probably derived from perillic acid [Poon *et al.*, 1996; Crowell *et al.*, 1994; Vigushin *et al.*, 1998]. Limonene (unchanged) and perillic acid artifacts (methyl ester) were also detected as minor plasma metabolites [Poon *et al.*, 1996]. Peak plasma levels for all metabolites were achieved 4-6 hours after administration, with the exception of limonene-8,9-diol which reached its peak level one hour after administration [Crowell *et al.*, 1994]. Phase II glucuronic acid conjugates have been identified in the urine of human volunteers for all major and minor metabolites. They include the glucuronic acid conjugates of perillic acid, dihydroperillic acid, limonene-8,9-diol, limonene-10-ol, limonene-6-ol, and limonene-7-ol (perillyl alcohol) [Poon *et al.*, 1996; Kodama *et al.*, 1974; 1976].

2.5.4 Metabolism in Rats and Other Animals

Similar to humans, the C_1 methyl substituent of limonene is oxidized in the rat to form perillic acid (see Figure 1). Perillic acid can then be excreted in the urine unchanged or as the glycine or glucuronic acid conjugate or it can be further oxidized to perillic acid-8,9-diol or 2-hydroxy-*p*-menth-8-en-7-oic acid. Approximately 85% of the urinary limonene metabolites in the rat were identified as perillic acid or a metabolite of perillic acid [Kodama *et al*., 1976]. Minor pathways reported for the rat include epoxidation of either the 1,2- or the 8,9- double bond, and subsequent hydrolysis to the diol.

Figure 1. Metabolism of *d*-limonene in Animals



Upon incubation with rat liver microsomes, the majority of *d*-limonene was converted to the 8,9-diol *via* its precursor the 8,9-epoxide and to a lesser extent, the 1,2-epoxide. Further

evidence of conversion of the 8,9-diol *via* the 8,9-epoxide was provided when 3,3,3-trichloropropene-1,2-oxide, an inhibitor of epoxide hydrolase, completely blocked the NADPH-dependent microsomal hydrolysis of the 8,9-epoxide to the 8,9-diol as shown by the accumulation of the 8,9-epoxide in the reaction medium. Epoxidation of the C_8-C_9 double bond is favored over epoxidation of the C_1 double bond, due to the steric hindrance of the 1-methyl group. The 1,2-epoxide underwent a very low rate of microsomal hydrolysis (1% of the rate for the 8,9-epoxide) which explains the absence of the 1,2 diol as a microsomal metabolite [Watabe *et al.*, 1981].

Incubation of male rat liver microsomes with either *d*-or *l*-limonene resulted in the formation of the corresponding perillyl alcohol and carveol stereoisomers. Cytochrome P-450 2C11 catalyzes formation of the alcohol metabolites. However, incubation of limonene with female rat liver microsomes resulted in lower activity for conversion to either alcohol. Use of phenolbarbital induced liver microsomes resulted in an increase in carveol metabolites. With fetal liver microsomes, rates of limonene hydroxylation were low or undetectable. After birth, limonene hydroxylation increased in males but not in females, and the formation of perillyl alcohol increased the most rapidly. The authors concluded that sex-related differences in metabolism may provide a basis for understanding the sex-specific chronic nephrotoxicity (see 3.4.4 Repeat Dose Toxicity) reported in Sprague Dawley rats [Miyazawa *et al.*, 2002].

In male rats orally administered 3 mmol/kg (408 mg/kg) of [C^{14}]-*d*-limonene, the 1,2-epoxide (82%), the 1,2-diol (5%), and *d*-limonene (13%) were detected in the renal proximal tubular cells where they were reversibly (40%) associated with *alpha*-2-globulin, a hepatic protein filtered by the glomeruli. It has been determined that these protein-*d*-limonene metabolite associations in the P-2 section of the proximal tubule are a prerequisite for the observed nephrotoxicity in the male rat [Lehman-McKeeman *et al*., 1989]. No such *alpha*-2-globulin has been observed in man [Olson *et al*., 1990].

Urinary metabolites isolated from male rabbits orally administered [^{14}C]-*d*-limonene included perillic acid-8,9-diol (major), *p*-menth-1,8-dien-10-ol, *p*-menth-1-ene-8,9-diol, perillic acid, *p*-

mentha-1,8-dien-10-yl glucuronic acid and 8-hydroxy-*p*-menth-1-en-9-yl- *beta*-glucopyranosiduronic acid [Kodama *et al.*, 1974].

Similar to limonene, myrcene participates in the epoxidation pathways. In male rabbits given 400-700 mg/kg bw [^{14}C]- *beta*-myrcene by gavage, the principal urinary metabolites identified were myrcene-3,10-glycol (40.7%), myrcene-1,2-glycol (20.8%), and uroterpenol (11.8%), illustrating that myrcene may undergo epoxidation of the 3,10-double bond (terminal double bond), epoxidation of the 1,2-double bond, or ring closure to uroterpenol, presumably *via* limonene that was derived from myrcene in the acidic conditions of rabbit stomachs. Similarly to data for *d*-limonene, epoxidation of the 3,10-double bond was favored over epoxidation of the 1,2-double bond [Ishida *et al*., 1981].

In Phase I metabolism, the biotransformation of *d*-limonene and myrcene as well as the other category members are catalyzed by NADPH-dependent cytochrome P450 (CYP). *d*-Limonene (monocyclic hydrocarbon), and *beta*-myrcene (acyclic hydrocarbon) have been shown to be substrates (upon repeated administration) and competitive inhibitors of the same isoenzyme, specifically CYP2B1 and CYP2C11 [Miyazawa *et al.*, 2002] providing evidence that the inclusion of the acyclic hydrocarbon *beta*-myrcene in this group is appropriate. In a study of the induction of liver monooxygenase by *beta*-myrcene, liver microsomes from female rats treated *via* gavage with 1000 mg/kg bw/d *beta*-myrcene for one or three days were isolated. Activities of several markers of different cytochrome P450 enzymes were monitored including pentoxyresorufin-O-depentylase (PROD) and benzyloxy-resorufin-O-dealkylation (BROD), which are selective markers of CYP2B1. *beta*-Myrcene produced marked increases in the activities of both PROD and BROD. Levels of apoproteins CYP2B1/2B2 were increased 8.2 fold after repeated treatment with *beta*-myrcene. [De-Oliveira *et al.*, 1997a]. Limonene has also been shown to induce the members of the CYP2B family in several studies [Maltzman *et al.*, 1991; Hiroi *et al.*, 1995].

Both *d*-limonene and *beta*-myrcene, when incubated with liver microsomes from phenobarbital treated rats at concentrations of 0.05-2 *micro*M for *d*-limonene and 0.02-1 microM for *beta*-

myrcene, produced a concentration-dependent reversible inhibition of PROD [De-Oliveira *et al.*, 1997b].

Humans are continually exposed to limonene, myrcene and the other members throughout their lifetimes, *via* consumption of a traditional diet or inhalation of air. Extensive studies on *d*-limonene show rapid metabolism to polar oxidized metabolites, followed by conjugation and rapid excretion. *beta*-Myrcene has been shown to undergo similar pathways of metabolism and to induce the same cytochrome P450 enzymes (CYP2B1) as *d*-limonene, so it is appropriate that these monoterpene hydrocarbons and their structural analogs be evaluated in the same chemical category. The remaining substances in this category are expected to undergo similar pathways of metabolism given the close structural similarity.

Based on the pharmacokinetic, biochemical and metabolic data, it is concluded that members of this chemical category exhibit similar chemical and biochemical fate. The monoterpene substances in this group undergo detoxication *via* Phase I metabolism by CYP450 isoenzymes followed primarily by conjugation with glucuronic acid in Phase II metabolism and excretion in the urine. The physiochemical and toxicological properties of these substances are consistent with their known reactivity and common metabolic fate.

3 Test Plan

3.1 Chemical and Physical Properties

3.1.1 Melting Point

All the substances in this chemical category are liquids at ambient temperature. The melting point of *d*-limonene is reported to be –74.35°C [CRC Handbook of Chemistry and Physics, 1986; IPCS, 1998]. The reported melting point for *dl*-limonene is -97 °C [CRC Handbook of Chemistry and Physics, 1986]. The calculated melting points for *d*-limonene, *dl*-limonene, terpinolene, myrcene, and dihydromyrcene are in the range from –29.5 to -66.1 °C [MPBPVPWIN EPI Suite, 2000].

3.1.2 Boiling Point

Literature values from recognized sources are available for limonene (*d* or *dl*) (178°C @ 760 mmHg), terpinolene (186°C @ 760 mmHg), myrcene (167°C @ 760 mmHg), dihydromyrcene (165-168°C @ 760 mmHg) [CRC Handbook of Chemistry and Physics, 1986] and *d*-limonene (175.5-176°C @ 760 mmHg) and myrcene (10°C at 44 mmHg) [Merck Index, 1996]. Measured values reported for limonene (176°C @ 760 mmHg), terpinolene (185°C @ 760 mmHg), myrcene (172°C @ 760 mmHg) [FMA] and dihydromyrcene (168°C @ 760 mmHg) [CRC Handbook of Chemistry and Physics, 1986] are consistent with standard literature resource values. Additionally, the measured boiling point for sweet orange oil (176°C @ 760 mmHg) that contains greater than 90% limonene [Givaudan-Roure, 1991h] is in good agreement with the boiling point for limonene itself. There is excellent agreement between boiling points reported in the literature and measured values for each of the four substances in this chemical category.

Of the seven mixtures in this chemical category, sweet orange oil, terpenes and terpenoids, sweet orange oil, and terpenes and terpenoids, limonene fraction, being composed almost exclusively of limonene, should exhibit boiling points close to that for limonene itself. Of the

remaining mixtures that contain mainly $C_{10}H_{16}$ hydrocarbons; boiling points should be somewhat lower than those reflected by major constituents of the mixture. Similar conclusions can be reached for the boiling point of the volatile portion of terpenes and terpenoids, turpentine oil residue. The boiling point of the remaining mixture (terpenes and terpenoids, turpentine oil, limonene fraction, distillation residues, being a complex mixture of terpene hydrocarbons and oxygenated terpene hydrocarbons) is undefined.

3.1.3 Vapor Pressure

The measured vapor pressure values for *d*-limonene (1.43 mm Hg or 0.19 kPa at 20°C) [IPCS, 1998] is similar to the calculated vapor pressure (1.2 mm Hg or 0.16 kPa at 20 °C) reported elsewhere [FMA]. The calculated and measured vapor pressures for limonene are in good agreement with calculated values for isomeric and homologous monoterpene hydrocarbons (terpinolene, 0.5 mm Hg or 0.07 kPa at 20 °C; *beta*-myrcene, 1.5 mm Hg or 0.2 kPa at 20 °C; sweet orange peel oil, 0.9 mm Hg or 0.12 kPa at 20 °C) [FMA]. Model values [MPBPVPWIN EPI Suite, 2000] for *d*-limonene (1.59 mm Hg), *dl*-limonene (1.63 mm Hg), terpinolene (1.44 mm Hg), myrcene (2.4 mm Hg), dihydromyrcene (2.6 mm Hg) are closely correlated with other calculated and the measured values. Based on the above data the vapor pressures of the five monoterpene hydrocarbons fall in the range from 0.5 to 2.6 mm Hg. Vapor pressures for the remaining monoterpenoid hydrocarbon mixtures and the volatile portion of the monoterpene hydrocarbon mixture in this category are expected to fall within this range.

3.1.4 Octanol/Water Partition Coefficients

Measured log Kow values using OECD 117 guidelines are available for two substances. Both terpinolene and sweet orange oil exhibit log Kow values of 5.3 [Givaudan-Roure, 1996a; 1996b]. The calculated log Kow values as reported by Syracuse Research Corporation (SRC), for five chemical substances in this category are in the range from 4.83 to 4.88 [SRC], and are consistent with the experimentally measured values. The narrow range and the close agreements with the two measured values in this group indicate consistency and imply reliability. It is expected that the log Kow values for members of this category are in the range from 4.8-5.3.

3.1.5 Water Solubility

Measured values for limonene and terpenes and terpenoids, sweet orange oil are 13.8 mg/L at 25 °C [WSKOWWIN EPI Suite, 2000c] and 30 mg/L at 20 °C, respectively [Givaudan-Roure, 1991]. Experimental values for myrcene and terpinolene of 5.6 mg/L [WSKOWWIN EPI Suite, 2000a] and 9.5 mg/L [WSKOWWIN EPI Suite, 2000b] at 25 °C and 23 °C, respectively, are in good agreement with the value for limonene. Model calculated water solubility values for limonene (*d*- and *dl*) is 0.74 mg/L and the value for terpinolene, myrcene and dihydromyrcene is 1.87 mg/L, [WSKOWWIN EPI Suite, 2000d], indicating the conservative nature of the model predictions. The solubility of all members of this chemical category at 25 ^{o}C is expected to be in the range from 5 to 30 mg/L.

3.1.6 New testing required

No new testing required.

3.2 Environmental Fate and Pathways

3.2.1 Photodegradation

The calculated photodegradation half-lives for the structurally defined terpenoid hydrocarbons in this chemical category, are in the range from 0.884 to 0.64 hours [AOPWIN EPI Suite, 2000]. These calculations are based on measured rate constants for radical reactions of OH, O_3 and NO_3 with monterpene hydrocarbons in this category [AOPWIN EPI Suite, 2000]. The short half-lives are predicted based on the abundant presence of reactive allylic hydrogens on members of this chemical category. Therefore these figures can be considered reliable. The photodegradation model was not applied to the seven mixtures. These mixtures are principally composed of the structurally defined hydrocarbons in this chemical category. As such, the photodegradation of the volatile portion of these mixtures is represented by the calculated photodegradation rates of its constituents.

3.2.2 Stability in Water

No hydrolysis is possible for any of the materials in this group. All are expected to be stable in aqueous solution.

3.2.3 Biodegradation

Three GLP experimental studies evaluating biodegradability are available for this group of substances using standard OECD protocols. Additional studies in soil horizons obtained from coniferous and deciduous forests [Misra *et al.*, 1996] provide a broader perspective on the biodegradation of monoterpene hydrocarbons in the environment.

Terpinolene was found to be biodegradable in two studies. In one study of inherent biodegradability, 80% biodegradation was reported using an OECD 302C guideline protocol after 28 and 31 days [Rudio, 1998]. In a study of ready biodegradability, 62.1% biodegradation was reported using an OECD 301B protocol after 28 days [Birch, 1996]). One study of the ready biodegradability of terpinolene by OECD 301F reported it to be not readily biodegradable (51% after 28 days) [Rudio, 1997] but the authors concluded that the high vapor pressure and low water solubility of terpene hydrocarbons leads to volatilization in the upper parts of the test vessel, thereby, limiting aerobic biodegradation.

Additional studies in extracts and slurries prepared from soils of coniferous and deciduous forest indicate rapid and complete biodegradation of limonene and terpinolene in a closed bottle test. Soil extracts from coniferous and hardwood watersheds were added to sealed flasks containing oxygen-saturated media that were preconditioned with limonene or terpinolene for 24 hours. Limonene and terpinolene underwent 100% biodegradation after approximately 1 day in acclimated medium and after day 8 in non-acclimated medium. The authors concluded the limonene and terpinolene are completely degradable in extracts prepared from watershed soils of coniferous or deciduous forests [Misra *et al.*, 1996].

In the soil regimen biodegradation tests, both limonene and terpinolene were found to be completely biodegradable within 8 days in unacclimated medium. Terpinolene was also found to

be readily biodegradable in two separate OECD studies. Given the close structural similarity of the members of this chemical category, it is reasonable to predict ready biodegradation for chemical substances and 6 of the 7 mixtures in this chemical category. In the remaining mixture, the monoterpene hydrocarbon fraction in the mixture containing appreciable quantities of polymeric terpenes is also expected to be biodegradable.

3.2.4 Fugacity

Transport and distribution in the environmental were modeled using Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11 [Trent University, 1999]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log Kow. Where measured values were available, these were used, but where they were not, calculated data from the EPIWIN series of programs were used. Given the similarity of the physical properties of the substances in this group, it is not unexpected that they would be predicted to exhibit similar distribution in the environment. The value of these calculations must be evaluated in the context that the substances in this chemical category are products of plant biosynthesis and are, therefore, ubiquitous in the environment. The model does not account for the influence of biogenic production on partitioning in the environment nor does it take into account biodegradation. Therefore, the relevance of fugacity calculations for these substances is questionable.

3.2.5 New testing required

No new testing required.

3.3 Ecotoxicity

3.3.1 Acute Toxicity to Fish

The 96 hour experimental LC50 values for limonene and terpinolene using fathead minnows in a flow-through system were reported to be 0.702 and 0.720 mg/L, respectively [Broderius, 1990]. Calculated 96 hour ECOSAR values are 0.221 mg/L (model input values of log Kow = 5.3, mp = -74.3 ^{o}C, water solubility = 13.8 mg/L) for limonene (*d* and *dl*), 0.198 mg/L (model

input values of log Kow = 54.88, mp = -29 °C, water solubility = 9.5 mg/L) for terpinolene, 0.198 mg/L (model input values of log Kow = 4.88, mp = -64 °C, water solubility = 5.6 mg/L) for myrcene, and 0.201 mg/L (model input values of log Kow = 4.88, mp = -64 °C, water solubility = 5.6 mg/L) for dihydromyrcene [ECOSAR EPI Suite, 2000].

Good correlation exists between the experimental and calculated 96 hour LC50 values, although model 96 hour LC50 values are always lower than measured values for the chemically defined substances in this category. Since limonene, terpinolene and *beta*-myrcene are the principal constituents of the mixtures in this chemical category, it is reasonable that they should have acute fish toxicities in the range of 0.2 to 1.0 mg/L.

3.3.2 Acute Toxicity to Aquatic Invertebrates

Seven experimental values are available for three members of this chemical category. The 48 hour EC50 for limonene with *Daphnia pulex* is reported to be 69.6 mg/L [Passino and Smith, 1987]. However, since the solubility of limonene is 13.8 mg/L at 25 °C, the actual test concentration is not known. The 96 hour LC50 value for limonene with *Daphnia magna* was reported to be 0.577 mg/L (95% C.I. 0.496-0.672 mg/L) while the 96 hr EC50 was reported to be 0.421 mg/L. When these tests were repeated using limonene from a different supplier, the 96 hour LC50 was reported to be 0.924 mg/L [US EPA]. The 48 hour LC50 for myrcene with *Daphnia magna* was reported to be 31 mg/L and is also considered unreliable given limits of solubility [Waggy and Blessing, 1986]. For terpinolene, the 96 hour LC50 was reported to be 2.55 mg/L and the EC50 was reported to be 1.38 mg/L [US EPA]. In another acute toxicity experiment with limonene and myrcene using brine shrimp, the 48 hour LC50 values were determined to be 104.1 and 39.2 ppm (approximately 104.1 and 39.2 mg/L), respectively [Saleh *et al*., 1998]. Calculated 48 hour LC50 values are 0.496, 0.612, 1.147 and 0.263 mg/L for limonene, terpinolene, myrcene and dihydromyrcene, respectively [ECOSAR EPI Suite, 2000].

The experimental and calculated acute invertebrate toxicity values for these substances indicate that all of the five chemical substances and the seven mixtures that are primarily composed of

these substances should have acute aquatic invertebrate toxicities on the order of 0.5 - 3.0 mg/L.

3.3.3 Acute Toxicity to Aquatic Plants

Experimental data for limonene and terpinolene are available for this chemical category. Both limonene and terpinolene were subjected to static 96 hour toxicity tests using green algae at test concentrations of 1.81 and 3.38 mg/L, respectively. Neither limonene nor terpinolene showed any significant inhibition at those test concentrations [Broderius, 1990]. Calculated 96 hour EC50 values for aquatic plants (*i.e.*, green algae) are lower than experimental values and are 0.360, 0.441, 0.813, and 0.194 mg/L for limonene, terpinolene, myrcene, and dihydromyrcene, respectively. The model should be considered extremely conservative given that experimental values are an order magnitude greater than calculated values. Each of the members of this chemical category are expected to all have acute aquatic plant toxicity exceeding 1.81 mg/L.

3.3.4 New Testing Required

No new testing required.

3.4 Human Health Data

3.4.1 Acute Toxicity

Oral and dermal LD50 values for five members (limonene, myrcene, terpinolene, dihydromyrcene and sweet orange peel oil) of this chemical category indicate a low order of both oral and dermal toxicity. All rabbit and rat dermal, and mouse and rat oral LD50 values exceed 4,000 mg/kg with the majority of values greater than 5,000 mg/kg [Levenstein, 1975; Moreno, 1972a, 1972b, 1972c, 1972d, 1973a, 1973b, 1980b; Tsuji *et al*., 1975a; Paumgartten *et al*., 1990]. Based on these data, it is concluded that all of the members of this chemical category are of very low acute toxicity.

3.4.2 *In vitro* Genotoxicity

Mutagenicity/genotoxicity testing has been performed on three members of this chemical category, including a complete battery of *in vitro* genotoxicity tests using limonene. No evidence of mutagenicity was observed when limonene [Heck *et al.*, 1989; Florin *et al.*, 1980; Muller, 1993; Haworth *et al*., 1983] was incubated with *Salmonella typhimurium* (SAL) strains TA98, TA100, TA102, TA1535, TA1537, and TA1538 with and without S-9 metabolic activation at concentrations up to and including 150,000 microgm/plate. Limonene did not induce chromosomal aberrations when incubated with Chinese hamster ovary cells at a concentration of 50-500 microgm/ml [Anderson *et al.*, 1990], nor did it induce sister chromatid exchanges in Chinese hamster ovary cells at concentrations of 16.2-162 microgm/ml [Anderson *et al.*, 1990]. In a mouse lymphoma forward mutation assay, limonene was negative in L5178Y cells with and without S-9 metabolic activation up to a maximum concentration of 100 microgm/ml [Heck *et al.*, 1989; Myhr *et al.*, 1990]. When incubated with Syrian hamster embryo cells up to 100 microgm/ml or 3 mM, limonene did not induce statistically significant cell transformation [Pienta, 1980; Rivedal *et al.*, 2000]. The effects of limonene on gap junction intercellular communications were also tested at concentrations up to 1 mM in Syrian hamster embryo cells, and showed no effects [Rivedal *et al.*, 2000].

In an *in vitro* chromosome aberration test with human lymphocytes, myrcene did not induce chromosomal aberrations at concentrations up to 1000 microgm/ml with and without metabolic activation [Kauderer *et al*, 1991]. When incubated with Chinese hamster ovary cells in a V79-HPRT Gene Mutation Assay, myrcene was not mutagenic with or without metabolic activation [Kauderer *et al*, 1991]. In sister chromatid exchange (SCE) tests with human lymphocytes, myrcene did not induce sister chromatid exchanges at concentrations up to 1000 microgm/ml with or without metabolic activation [Kauderer *et al*, 1991]. Additionally, there was no evidence of genotoxicity when myrcene was incubated with V79 and hepatic tumor (HPT) Chinese hamster cells at concentrations up to 500 microgm/ml in SCE assays [Roscheisen *et al*, 1991]. In fact, myrcene reduced the SCE inducing effect of S-9 mix activated cyclophosphamide in human lymphocytes and Chinese hamster ovary (CHO) cells, and aflatoxin B1 in V79 and HTC Chinese hamster cells in a dose dependent manner [Kauderer *et al*, 1991; Roscheisen *et al.*, 1991].

No evidence of mutagenicity was observed when sweet orange peel oil was incubated with *Salmonella typhimurium* (SAL) strains TA98, TA100, TA1535, TA1537, and TA1538 with and without S-9 metabolic activation at concentrations up to 5000 microgm/plate [Heck *et al.*, 1989 and Crebelli *et al.*, 1990]. In a mouse lymphoma forward mutation assay, sweet orange peel oil was positive in L5178Y cells with and without S-9 metabolic activation up to a maximum concentration of 75 microgm/ml but only at highly toxic concentrations. The authors noted that that positive results in this assay may be associated with changes in physiologic culture conditions (pH and osmolality) [Heck *et al.*, 1989]. Negative results were obtained with sweet orange peel oil in the Rec DNA repair assay using *Bacillus subtilis* strains H17 and M45 [Kuroda *et al*., 1989].

In eighteen separate *in vitro* tests on the mutagenicity and genotoxicity of limonene, myrcene, and sweet orange peel oil; the majority was negative, with the exception of the mouse lymphoma assay using sweet orange peel oil. This result is questionable given the culture conditions present and the negative results of the mouse lymphoma assay using limonene since limonene is the majority (greater than 90%) constituent of sweet orange peel oil. It is reasonable

to conclude that given the structural similarity between the members of this chemical category, the substances in this category exhibit low genotoxic potential *in vitro*.

3.4.3 *In vivo* Genotoxicity

Two *in vivo* genotoxicity assays are available for two substances in this chemical category. In an *in vivo* mammalian spot test, no evidence of mutagenicity was reported when mouse embryos were treated *in utero* with 215 mg/g bw/d limonene on days 10 -11 of gestation [Fahrig, 1984].

In an *in vivo* cytogenetic bone marrow assay, *beta*-myrcene (100, 500 or 1,000 mg/kg) was orally administered *via* gavage to up to four male and female Wistar rats. Corn oil was used as the negative control while cyclophosphamide (30 mg/kg *via* intraperitoneal injection) was used as the positive control. A mitotic inhibitor (colchicine 5 mg/kg ip) was injected 1 hour before sacrifice. At 24 or 48 hours, animals were sacrificed and bone marrow cells harvested. Evaluations included the mitotic index and the frequency of chromosomal aberrations. A dose-related increase in the mitotic index in bone marrow cells was reported for rats administered the test substance. The authors commented that this might be an interaction between *beta*-myrcene, which is known to induce CYP-450 enzymes, and colchicine, which arrests cell division at metaphase. *beta*-Myrcene may have increased the bioavailability of colchicine leading to the increase in mitotic index observed in the experiment. No significant increases in chromosomal aberrations were reported in the treated animals at either 24 or 48 hours. The authors concluded that given the results, *beta*-myrcene was not clastogenic to the rat when orally administered at dose levels up to 1000 mg/kg bw [Zamith *et al*., 1993]. Based on the results of this *in vivo* genotoxicity assay and the numerous *in vitro* genotoxicity assays, it is unlikely that any of these materials would exhibit a significant genotoxic potential *in vivo*.

3.4.4 Repeat Dose Toxicity

Orange oil was administered intragastrically to female B6C3F1 mice daily for 5 days at dose levels of 0, 625, 1250 or 2500 mg/kg bw to determine effects on humoral and cell-mediated immune responses. A host resistance assay (Listeria monocytogenes bacterial challenge) was

used to assess cell-mediated immunity while the antibody plaque forming cell response to sheep erythrocytes was used to measure humoral immunity. Other parameters evaluated included body weights, lymphoid organ weights and spleen cellularity. Orange oil had no effects on cell-mediated or humoral immune response at any dose level tested [Gaworski *et al*., 1994].

Groups of five mice of each sex were administered 0, 413, 825, 1650, 3300, or 6600 mg/kg *d*-limonene in corn oil by gavage once per day for 12 days over a 16-day period. Animals were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals. All but one animal receiving 3300 or 6600 mg/kg bw/d limonene died within three days of study initiation. No treatment-related clinical signs were observed in mice receiving doses of 1650 mg/kg bw/d or lower [NTP, 1990].

Groups of five rats of each sex were administered 0, 413, 825, 1650, 3300, or 6600 mg/kg *d*-limonene in corn oil by gavage once per day for 12 days over a 16-day period. Animals were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals. All but two females receiving 3300 or 6600 mg/kg bw/d limonene died within two days of study initiation. No treatment related clinical signs were observed in rats receiving doses of 1650 mg/kg bw/d or lower [NTP, 1990].

Groups of five young adult male F344/N rats each were administered *d*-limonene at dose levels of 0, 75, 150 or 300 mg/kg bw/d five days a week for 27 days. Observations included daily body weight, weekly food intake, liver and kidney weights and light microscopy and histology of liver and kidneys. Rats were examined for hyaline droplet formation, granular cast formation and chronic nephrosis. Two-dimensional gel electrophoresis evaluation of protein profiles was conducted on samples of kidneys in the 150 mg/kg dose group killed on day 6. Dose related increases in liver and kidney weights were reported for all dose levels. Renal effects were noted including protein profile changes, hyaline droplet formation, and accumulation of *alpha*-2-

globulin was reported. Chronic nephrosis was present in all kidneys of treated animals killed on day 27 [Kanerva *et al*., 1987].

Groups of ten rats of each sex were administered 0, 150, 300, 600, 1200 or 2400 mg/kg bw/d *d*-limonene in corn oil by gavage once per day, five days a week for 13 weeks. Animals were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals. Histological examinations were performed on all vehicle control and high dose animals and all female rats in the 1200 mg/kg group. Tissues examined included adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), femur, sternebrae or vertebrae including marrow, gross lesions and tissue masses with regional lymph nodes, heart kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, small intestine, spinal cord (if neurologic signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Kidneys were examined for all male rats. Ninety percent of female rats (9/10) and fifty percent of male rats (5/10) receiving 2400 mg/kg bw/d limonene died within the first week of the study. The final mean body weights of male rats receiving the three highest doses (600, 1200 or 2400 mg/kg bw/d) were reported to be 6%, 12%, or 23% lower than that of the controls, respectively. Rough hair coats, lethargy, and excessive lacrimation were observed for all animals at the two highest dose levels. Nephropathy was reported for all groups of male rats but a dose related increase in severity of the lesion was reported for the dosed groups. The nephropathy was characterized by degeneration of epithelium in the convoluted tubules, granular casts with tubular lumens, primarily in the outer stripe of the outer medulla, and regeneration of the tubular epithelium. Hyaline droplets were observed in the epithelium of the proximal convoluted tubules in all groups of male rats including vehicle controls. Upon further review to determine if there were differences in these findings between control and treated animals, the blinded slides revealed no definite differences in the accumulation of hyaline droplets [NTP, 1990].

Groups of ten mice of each sex were administered 0, 125, 250, 500, 1000 or 2000 mg/kg bw/d *d*-limonene in corn oil by gavage once per day, five days a week for 13 weeks. Animals

were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals. Histological examinations were performed on all vehicle control and high dose animals. Tissues examined included adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), femur or sternebrae or vertebrae including marrow, gallbladder, gross lesions and tissue masses with regional lymph nodes, heart kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, small intestine, spinal cord (if neurologic signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. One of 10 males and 2/10 females administered 2000 mg/kg bw/d limonene and 1/10 females administered 500 mg/kg bw/d limonene died before the end of the study. Several other animals also died as a result of gavage error. Mean body weights were 10% lower than control for male mice and 2% lower than control for female mice for the two highest dose levels. An alveolar cell adenoma was reported in the lung of one female at the highest dose level. Clinical signs of rough hair coats and decreased activity were reported for the two highest dose levels [NTP, 1990].

Groups of five-week-old male rats received 0, 2, 5, 10, 30 or 75 mg/kg bw/d *d*-limonene daily *via* oral gavage for 13 weeks (5 days a week). Rats from selected dose groups were necropsied throughout the study (days 8-29), with all remaining rats necropsied at the end of the study. Rats were observed daily for toxicity signs. Body weights were taken daily. Linear regression analyses indicated increased relative kidney and liver weights at the two highest dose levels. Histological examination revealed changes characterized by hyaline droplet formation, granular casts and multiple cortical changes, all of which was classified as chronic nephrosis. Exacerbation of hyaline droplet formation was reported at the earliest necropsy eight days after administration at the 10 mg/kg bw/d dose level [Webb *et al.*, 1989].

d-Limonene was orally administered to Sprague-Dawley rats daily for 30 days at the following dose levels 0, 277, 554, 1385, or 2,770 mg/kg bw in order to investigate the effect on the fine structure of the liver, kidney and blood cells. No morphological changes of renal corpuscles and

tubular cells were observed. Some alterations were detected in the glomerular epithelium from the kidneys of rats treated at the highest dose level [Kodama *et al.*, 1977b, 1977c].

Groups of fifty male and fifty female rats each were administered 0, 75 or 150 mg/kg bw/d or 0, 300 or 600 mg/kg bw/d *d*-limonene, respectively, in corn oil by gavage once per day, five days a week for 103 weeks. Animals were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study; all vehicle control; all low dose female rats and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland. Tissues examined in low dose male rat groups included adrenal glands, kidney, liver, spleen, and testis. Mean body weights for male rats administered 150 mg/kg bw/d *d*-limonene were generally 4-7% lower than vehicle controls from week 2 to study termination. Mean body weights of high dose females were generally 4-7% lower than vehicle controls from week 28 to study termination. No treatment related clinical signs were reported for the duration of the study. Survival of the high dose male group was significantly greater than that of the vehicle alone after week 81. Survival of the high dose female group was significantly lower than that of the vehicle controls after week 39. In the kidneys of male rats, dose-related increases were observed in the incidence of mineralization and epithelial hyperplasia. A dose-related increase in the severity of spontaneous nephropathy was reported in male rats administered limonene. Increased incidences in tubular cell hyperplasia and neoplasia were also reported in dosed male rats. Tubular cell adenoma incidence in high dose male rats and tubular cell adenoma or tubular cell

carcinomas (combined) in dosed male rats were significantly greater than vehicle controls. The authors determined that under the conditions of these 2-year gavage studies there was clear evidence of carcinogenic activity of *d*-limonene for male F344/N rats as shown by increased incidences in tubular cell hyperplasia, adenomas, and adeno-carcinomas of the kidney. There was no evidence of carcinogenic activity of *d*-limonene for female rats receiving 300 or 600 mg/kg bw/d [NTP, 1990].

Groups of fifty male and fifty female mice each were administered 0, 250, or 500 mg/kg bw/d or 0, 500 or 1000 mg/kg bw/d *d*-limonene, respectively, in corn oil by gavage once per day, five days a week for 103 weeks. Animals were housed five per cage and fed *ad libitum*. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study, all vehicle controls, and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gallbladder, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland. Tissues examined in low dose groups include liver for female mice. Mean body weights for female mice administered 1000 mg/kg bw/d *d*-limonene were generally 5-15% lower than vehicle controls from week 28 to study termination. No treatment related clinical signs were reported for the duration of the study. Survival of the low dose male group was significantly lower than that of the vehicle controls by study termination. The authors determined that under the conditions of these 2-year gavage studies there was no evidence of carcinogenic activity of *d*-limonene for male or female B6C3F1 mice at the dose levels tested [NTP, 1990].

Evaluation of results of the NTP bioassay of d-limonene in male F344/N rats

It has been demonstrated that renal lesions, which were observed in the NTP study, resulted from the accumulation of aggregates of *alpha*-2 microglobulin (a low molecular-weight protein synthesized in the liver) and limonene-1,2-epoxide in the P2 segment of the renal proximal tubule. These aggregates prevent lysosomal degradation, which leads to accumulation in the cytoplasm of the protein or the protein-chemical complex, which leads to single cell necrosis, and ultimately, renal neoplasia (Lehmann-McKeeman *et al.*, 1990; Hildebrand *et al.*, 1997). This phenomenon has only been observed in the male F344/N rat (Strasser, 1988; Borghoff *et al.*, 1990).

The gene that encodes *alpha*-2 microglobulin has been isolated and the sequence deduced (Untermann *et al.*, 1981). These proteins are expressed in the liver under hormonal control (Roy and Neuhaus, 1967; Wang and Hodgetts, 1998). *alpha*-2 microglobulin belongs to the *alpha*-2 microglobulin super family of proteins that are characterized by a unique hydrophobic binding pocket. The lesions do not develop in the female F344/N rat or in humans (Bucher *et al.*, 1986). Subsequent investigations have shown that the *alpha*-2 microglobulin nephropathy found in the F344/N male rat only develops in mammals that express the hepatic form of *alpha*-2 microglobulin (Swenberg, 1989) unlike other strains of rats (Dietrich and Swenberg, 1991), mice (Bucher *et al.*, 1986; Lehman-McKeeman, 1994) and dogs (Webb, 1990).

Transgenic mice that express rat *alpha*-2 microglobulin were tested for their ability to form hyaline droplets and develop nephropathies similar to their adult male rat counterparts (Lehman-McKeeman and Caudill, 1994). This study involved male F344 rats as positive control, transgenic C57BL/6J mice as experimental group and native C57BL/6 mice as negative controls. The animals at age 70-75 days were placed in metabolic cages and received 150 mg/kg bw per day *d*-limonene in corn oil by gavage for three days. Limonene was used to induce renal nephropathy in adult male rats, as it was shown to be a potent inducer in the NTP studies (EPA, 1991) [NTP, 1990]. Twenty-four (24) hours after the last dose, the animals were sacrificed and the kidneys analyzed for evidence of nephropathy. Hyaline droplet formation was evaluated on a subjective scale, size and intensity (0-4) multiplied by tubular loading (0-3) for an overall scale of 0-12 with 12 being the most severe. In the absence of *d*-limonene, the control

groups transgenic mice and rats showed a hyaline droplet score of 1+/- 0 and 6 +/- 0.5, respectively. The test transgenic mice and rats showed a hyaline droplet score of 2.5 +/- 0.3 and 11 +/- 1.3, respectively upon dosing with *d*-limonene. The native mice developed no signs of hyaline droplet formation and tested negative for presence of *alpha*-2 microglobulin in their urine. The authors assert that based on the data presented "*alpha*-2 microglobulin is the only protein that is involved in the etiology of hyaline droplet nephropathy".

An increase in the kidney-type-*alpha*-2 microglobulin was seen in the urine of male Sprague-Dawley rats when these animals were administered greater than 30 mg/kg/day of *d*-limonene for 7 days by gavage. The increases in the urinary kidney-type-*alpha*-2 microglobulin are dose-dependent and parallel-elevated accumulation in the kidney cells (Saito, 1996).

While humans produce low molecular weight serum proteins, which are reabsorbed by the kidney, there is no evidence that *alpha*-2 microglobulin is produced (Olson, 1990). Urine collected from adult male F344 rats and humans revealed no evidence indicative of *alpha*-2 microglobulin production in humans (Olson, 1990).

It is unknown whether any human serum proteins possess a binding site similar to that of *alpha*-2 microglobulin. Although this is a possibility, it appears remote, since female rats and mice do not show the renal changes noted in male rats exposed to limonene. It should be noted that there is a class of human proteins referred to as the *alpha*-2 microglobulin related proteins. They appear to have no functional relationship to the adult male rat urine proteins. The human protein has a higher molecular weight, 25 kDa and is a component of a neutrophil gelatinase complex (Kjeldsen *et al.*, 2000; Triebel *et al.*, 1992). An extensive review of the current scientific literature and genome databases reveals no native protein or biological entity that acts as a nephropathy agent like mature male rat *alpha*-2 microglobulin. The accumulated evidence indicates that it is the unique anatomical, physiological, and biochemical properties of the male rat kidney, especially the proximal convoluted tubule, that allows *d*-limonene to interfere with renal processing of the strain-specific *alpha*-2 microglobulin. Therefore, this process is not predictive of human carcinogenicity. In a comprehensive review of *alpha*-2 microglobulin

nephropathy and associated renal tubule tumors produced in the male F344/N rat exposed to limonene and other simple chemical substances (e.g. isophorone, decalin and methyl isobutyl ketone), it was concluded that the F344/N rat is not an appropriate model for assessing human renal carcinogenic risk (EPA, 1991). After careful review, it has been concluded that the mechanisms leading to the renal carcinogenic findings in the F344/N male rat are largely known and strongly indicate that the nephropathy associated with *d*-limonene have no significance for human risk assessment (Burdock *et al.*, 1990).

Groups of ten Sprague Dawley rats of each sex were administered 0, 240, 600 or 1500 mg/kg bw/d sweet orange oil in 1% methyl cellulose by gavage daily for 30 days. Observations included survival, clinical observations, body weights, food consumption, clinical pathology, gross pathology, organ weights and histopathology. No treatment related effects were reported for survival, clinical observations, body weights or food consumption. Decreases in glucose levels related to treatment were reported in the mid-dose females and high-dose males and females. Increases in serum albumin and total serum protein were observed in all treated females and the high-dose males. Histopathology revealed treatment related lesions in the nonglandular stomach of the high-dose males and females and in the kidney of all treated male groups. Kidney weights were also increased in all of the treated male groups and in the high-dose female group. Liver weight increases related to treatment were reported for the high-dose females and all treated male groups. The authors concluded that the no-observed-effect-level (NOEL) under conditions of this study was less than 240 mg/kg bw/d for both male and female rats. The authors noted that the kidney changes observed in the male rat at all dose levels were expected given the known interaction between limonene and *alpha*-2-microglobulin (Serota, 1990). Limonene is the principal constituent of orange oil [Bauer and Garbe, 1985].

Several repeat dose studies have been conducted and demonstrate that this category of monoterpenoid hydrocarbons is of low toxic potential. The kidney changes seen in male rats administered limonene have been well characterized, and are known to be specific to the male rat and of no significance in human risk assessment.

3.4.5 Reproductive Toxicity

beta-Myrcene was administered *via* gavage to female Wistar rats at dose levels of 250, 500, 1000 and 1500 mg/kg bw/d of *beta*-myrcene from the 15th day of gestation until weaning of the offspring which was day 21 postnatal. Reproductive capacity was assessed in the exposed offspring upon reaching maturity (120 days). Mortality, weight gain and post-natal development were evaluated (see section 3.4.6 Developmental/Teratogencity Toxicity below for developmental effects) Fertility was impaired in female offspring exposed to 1000 or 1500 mg/kg bw/d of *beta*-myrcene [Delgado *et al*., 1993b].

Three experimental groups (15 male and 45 female Wistar rats per group) were administered *beta*-myrcene dissolved in peanut oil *via* gavage at dose levels of 0, 100, 300, or 500 mg/kg bw/d. The exposure period was 91 days prior to and during the mating period for the males and 21 days prior to and during the mating period for females, pregnancy, and lactation until 21 days post parturition. All parent animals were evaluated for weight development, mortality, and toxicity signs. Pregnant females were also evaluated for weight gain, spontaneous abortions, dystocia and prolonged duration of pregnancy. All males were sacrificed and decapitated at the conclusion of mating. One third of the females in each dose group were sacrificed at day 21 of pregnancy. All fetuses were examined for skeletal abnormalities. After the remaining pregnant females gave birth, the offspring were weighed and examined for development, specifically, incisor eruption, fur, downy hair, and eye opening. At weaning on day 21, all mothers were sacrificed and necropsied.

Neither deaths nor signs of toxicity were reported in male rats at any dose level. No statistically significant differences in body weight gain were reported between control and test animals. A slight increase in liver and kidney weights was reported for treated male (highest dose only) and female rats. No morphological alterations of the liver or testis tissue were revealed upon microscopic examination. No effects were reported on the number of spermatids in the testis or on the number of spermatozoa in the cauda epididymis. No adverse effects on body weight gain and no other signs of toxicity were observed in treated female rats during the pre-mating or

mating periods. No treatment related effects were reported on fertility as measured by the mating index and pregnancy index upon comparison to controls. At the highest dose level, a slight increase in the resorption rate and a parallel decrease in the ratio of live fetuses per implantation site were reported.

Increases in the occurrence of fetal skeleton abnormalities between control and treated groups were reported at the 500 mg/kg bw/d level. No adverse effects were reported on duration of pregnancy, labor, pup mortality, and maternal or offspring weight changes. Slight delays in incisor eruption (300 mg/kg bw/d) and eye opening (100, 300 mg/kg bw/d) were reported but were not dose-related. The authors attributed the increase in skeletal abnormalities at the highest dose level tested to known strain-specific anomalies including increases of dislocated sternums, and lumbar extra ribs. The authors concluded that the NOAEL for toxic effects on fertility and general reproductive performance *via* the oral route was 300 mg /kg bw/d for *beta*-myrcene [Paumgartten *et al.*, 1998].

Groups of ten female rats were orally administered sweet orange oil *via* gavage at dose levels of 0, 375, 750 or 1500 mg/kg bw/d for seven days prior to and through cohabitation, gestation, delivery and a four day lactation period. The vehicle was corn oil. Body weight, food consumption and clinical signs were recorded throughout the observation period. All dams were necropsied and examined for gross lesions on Day 25 of presumed gestation for rats not delivering a litter and four days postpartum for rats delivering a litter. Pups delivered were sacrificed on day 4 postpartum, any pups dying during the lactation period were necropsied. No deaths occurred at any dose level. Statistically significant numbers of rats from all three dose groups experienced excess salvation during the pre-mating and gestation periods, and during the lactation period for high-dose animals. The dosed rats had decreased weight gains compared to the control rats during the seven day premating period. Absolute and relative maternal food consumption was significantly decreased for the 750 and 1500 mg/kg bw/d dose groups during the seven day premating period. No treatment related effect on mating performance or fertility was reported at any dose level. A significant increase in stillbirths and pup deaths was reported for the highest dose group compared to the control group (See 3.4.6

Developmental/Teratogencity Toxicity below for developmental effects) [Hoberman *et al.*, 1989].

Given the results of three reproductive toxicity assays using sweet orange peel oil predominantly composed of *d*-limonene and *beta*-myrcene, it may be concluded that the substances within this chemical category exhibit low reproductive toxicity potential.

3.4.6 Developmental/Teratogenicity Toxicity

Four groups of twenty Wistar female rats each were administered 0, 591 or 2,869 mg/kg bw/d *d*-limonene on days 9-15 of gestation. At the highest dose level, increases in maternal mortality and decreases in maternal and fetal body weights were reported. Additionally at the highest dose level, delayed ossification of fetal metacarpal bones and proximals phalanx and decreased weights of the thymus, spleen, and ovaries were reported. The NOAEL for both maternal and offspring toxicity was reported to be 591 mg/kg bw/d [Tsuji *et al.*, 1975b].

Pregnant Japanese white rabbits were administered 0, 250, 500 or 1,000 mg/kg bw/d *d*-limonene on days 6 to 18 of gestation. Increased maternal mortality was reported at the highest dose level. Significant decreases in maternal body weight gain and food consumption were temporarily observed at the 500 and 1,000 mg/kg bw/d dose levels. No treatment related effects were reported for the offspring. The NOAEL for maternal toxicity was reported to be 250 mg/kg bw/d. The NOAEL for offspring toxicity was reported to be greater than 1,000 mg/kg bw/d [Kodama *et al.*, 1977a].

Pregnant ICR mice were administered 0, 591 or 2,363 mg/kg bw/d *d*-limonene on days 7 to 12 of gestation. Significant decreases in body weight gain were reported for pregnant ICR mice administered the highest dose level of *d*-limonene. In the offspring, increased incidence of fused ribs compared to that of the controls, delayed ossification of some bones and decreased body weight gain were reported at the highest dose level tested. The NOAEL for both maternal and offspring toxicity was reported to be 591 mg/kg bw/d [Kodama *et al.*, 1977a].

Pregnant Wistar rats were administered 0, 250, 500 or 1,200 mg/kg bw/d *beta*-myrcene on gestational days 6-15. The vehicle was corn oil. Decreased maternal weight gain was reported at the 1,200 mg/kg bw/d dose. Increased fetal skeletal malformations were reported at the 1,200 mg/kg bw/d dose level. The NOAEL for both maternal and offspring toxicity was reported to be 500 mg/kg bw/d [Delgado *et al.*, 1993a].

beta-Myrcene was administered *via* gavage to female Wistar rats at dose levels of 250, 500, 1,000 or 1,500 mg/kg bw/d from pregnancy day 15 until weaning of the offspring, which was day 21 postnatal. Mortality, weight gain and post-natal development were evaluated. Reproductive capacity was assessed in the exposed offspring upon reaching maturity (120 days). No adverse effects were noted in the offspring at the lowest dose level tested. Decreased body weight, increased perinatal mortality, and delayed developmental landmarks were noted at the 500, 1000 and 1500 mg/kg bw/d dose levels. Fertility was impaired in female offspring exposed to the two highest doses of *beta*-myrcene. The NOAEL for peri- and post-natal development was set at 250 mg/kg bw/d [Delgado *et al.*, 1993b].

Groups of ten female rats were orally administered sweet orange oil *via* gavage at dose levels of 0, 375, 750 or 1,500 mg/kg bw/d for seven days prior to and through cohabitation, gestation, delivery and a four day lactation period. The vehicle was corn oil. Body weight, food consumption and clinical signs were recorded throughout the observation period. All dams were necropsied and examined for gross lesions on day 25 of presumed gestation for rats not delivering a litter and four days postpartum for rats delivering a litter. Pups delivered were sacrificed on day 4 postpartum, any pups dying during the lactation period were necropsied. No deaths occurred at any dose level. Statistically significant numbers of rats from all three dose groups experienced excess salvation during the premating and gestation periods, and during the lactation period for high-dose animals. The dosed rats had decreased weight gains compared to the control rats during the seven-day premating period. Absolute and relative maternal food consumption was significantly decreased for the 750 and 1500 mg/kg bw/d dose groups during the seven day premating period. No treatment related effects were reported on maternal body weight, changes in body weight, and absolute and relative feed consumption during the lactation

period. No treatment related effect on mating performance or fertility was reported at any dose level. A significant increase in stillbirths and pup deaths was reported for the highest dose group compared to the control group. The treatment with sweet orange oil had no effect on the incidence of malformations or gross lesions in the pups. The NOAEL for administration of sweet orange peel oil under the conditions of this study was reported to be less than 375 mg/kg bw/d for maternal toxicity and 750 mg/kg bw/d for offspring development [Hoberman *et al*., 1989].

Given the results of six developmental toxicity assays using limonene, sweet orange oil and *beta*-myrcene, it may be concluded that the substances within this chemical category exhibit low developmental toxicity potential.

3.4.7 New Testing Required

No new testing required.

3.5 Test Plan Table

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
CAS No. 5989-27-5(*d*-) **CAS No. 138-86-3** (*dl*-) Limonene	**A**	**A**	**A, Calc**	**Calc**	**A**
CAS No. 586-62-9 Terpinolene	**Calc**	**A**	**Calc**	**A, Calc**	**A**
CAS No. 123-35-3 Myrcene	**Calc**	**A**	**Calc**	**Calc**	**A**
CAS No. 2436-90-0 Dihydromyrcene	**Calc**	**A**	**Calc**	**Calc**	**Calc**
CAS No. 68956-56-9 Hydrocarbons, terpene processing by-products (require constituents)	**NA**	**R**	**NA**	**NA**	**NA**
CAS No. 8008-57-9 Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	**NA**	**A**	**NA**	**NA**	**NA**
CAS No. 68647-72-3 Terpenes & terpenoids, sweet orange oil	**NA**	**A**	**Calc**	**A**	**A**
CAS No. 65996-99-8 Terpenes & terpenoids, turpentine oil, limonene fraction	**NA**	**R**	**NA**	**NA**	**NA**
CAS No. 65996-98-7 Terpenes & terpenoids, limonene fraction	**NA**	**R**	**NA**	**NA**	**NA**
CAS No. 68334-40-7 Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue	**NA**	**R**	**NA**	**NA**	**NA**
CAS No. 68938-00-1 Terpenes & terpenoids, turpentine-oil residue	**NA**	**R**	**NA**	**NA**	**NA**

Chemical	Environmental Fate and Pathways			
	Photodegra-dation	Stability in Water	Biodegra-dation	Fugacity
CAS No. 5989-27-5(*d*-) **CAS No. 138-86-3** (*dl*-) Limonene	**Calc**	**NA**	**A**	**Calc**
CAS No. 586-62-9 Terpinolene	**Calc**	**NA**	**A**	**Calc**
CAS No. 123-35-3 Myrcene	**Calc**	**NA**	**R**	**Calc**
CAS No. 2436-90-0 Dihydromyrcene	**Calc**	**NA**	**R**	**Calc**
CAS No. 68956-56-9 Hydrocarbons, terpene processing by-products (require constituents)	**R**	**NA**	**R**	**R**
CAS No. 8008-57-9 Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	**R**	**NA**	**R**	**R**
CAS No. 68647-72-3 Terpenes & terpenoids, sweet orange oil	**R**	**NA**	**R**	**R**
CAS No. 65996-99-8 Terpenes & terpenoids, turpentine oil, limonene fraction	**R**	**NA**	**R**	**R**
CAS No. 65996-98-7 Terpenes & terpenoids, limonene fraction	**R**	**NA**	**R**	**R**
CAS No. 68334-40-7 Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue	**R**	**NA**	**R**	**R**
CAS No. 68938-00-1 Terpenes & terpenoids, turpentine-oil residue	**R**	**NA**	**R**	**R**

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
CAS No. 5989-27-5(*d*-) **CAS No. 138-86-3** (*dl*-) Limonene	**A, Calc**	**A, Calc**	**A, Calc**
CAS No. 586-62-9 Terpinolene	**A, Calc**	**A, Calc**	**A, Calc**
CAS No. 123-35-3 Myrcene	**Calc**	**A, Calc**	**Calc**
CAS No. 2436-90-0 Dihydromyrcene	**Calc**	**Calc**	**Calc**
CAS No. 68956-56-9 Hydrocarbons, terpene processing by-products (require constituents)	**R**	**R**	**R**
CAS No. 8008-57-9 Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	**R**	**R**	**R**
CAS No. 68647-72-3 Terpenes & terpenoids, sweet orange oil	**R**	**R**	**R**
CAS No. 65996-99-8 Terpenes & terpenoids, turpentine oil, limonene fraction	**R**	**R**	**R**
CAS No. 65996-98-7 Terpenes & terpenoids, limonene fraction	**R**	**R**	**R**
CAS No. 68334-40-7 Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue	**R**	**R**	**R**
CAS No. 68938-00-1 Terpenes & terpenoids, turpentine-oil residue	**R**	**R**	**R**

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
CAS No. 5989-27-5(*d*-) **CAS No. 138-86-3** (*dl*-) Limonene	**A**	**A**	**A**	**A**	**R**	**A**
CAS No. 586-62-9 Terpinolene	**A**	**R**	**R**	**R**	**R**	**R**
CAS No. 123-35-3 Myrcene	**A**	**A**	**A**	**R**	**A**	**A**
CAS No. 2436-90-0 Dihydromyrcene	**A**	**R**	**R**	**R**	**R**	**R**
CAS No. 68956-56-9 Hydrocarbons, terpene processing by-products (require constituents)	**R**	**R**	**R**	**R**	**R**	**R**
CAS No. 8008-57-9 Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	**A**	**A**	**R**	**A**	**A**	**A**
CAS No. 68647-72-3 Terpenes & terpenoids, sweet orange oil	**R**	**R**	**R**	**R**	**R**	**R**
CAS No. 65996-99-8 Terpenes & terpenoids, turpentine oil, limonene fraction	**R**	**R**	**R**	**R**	**R**	**R**
CAS No. 65996-98-7 Terpenes & terpenoids, limonene fraction	**R**	**R**	**R**	**R**	**R**	**R**
CAS No. 68334-40-7 Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue.	**R**	**R**	**R**	**R**	**R**	**R**
CAS No. 68938-00-1 Terpenes & terpenoids, turpentine-oil residue	**R**	**R**	**R**	**R**	**R**	**R**

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
T	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

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WSKOWWIN EPI Suite (2000d) US Environmental Protection Agency.

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AR201-13756B

The Flavor and Fragrance High Production Volume Consortia

The Terpene Consortium

Robust Summaries for Monoterpene Hydrocarbons

d-Limonene	CAS No. 5989-27-5
dl-Limonene	CAS No. 138-86-3
Terpinolene	CAS No. 586-62-9
Myrcene	CAS No. 123-35-3
Dihydromyrcene	CAS No. 2436-90-0
Hydrocarbons, terpene processing by-products	CAS No. 68956-56-9
Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)	CAS No. 8008-57-9
Terpenes & terpenoids, sweet orange oil	CAS No. 68647-72-3
Terpenes & terpenoids, turpentine oil, limonene fraction	CAS No. 65996-99-8
Terpenes & terpenoids, limonene fraction	CAS No. 65996-98-7
Terpenes & terpenoids, turpentine oil, limonene fraction, distillation residue	CAS No. 68334-40-7
Terpenes & terpenoids, turpentine-oil residue	CAS No. 68938-00-1

FFHPVC Terpene Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

The Flavor and Fragrance High Production Volume Chemical Consortia

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The Flavor and Fragrance High Production Volume Consortia

Robust Summaries for Monoterpene Hydrocarbons

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 Chemical and Physical Properties

1.1 Melting Point

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Measured
Melting Point	-74.35 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	CRC Handbook of Chemistry and Physics (1986) 67th edition, Robert C. Weast, editor, The Chemical Rubber Co Press, Inc. Boca Raton, Florida.

Substance Name	*dl*-Limonene (dipentene)
CAS No.	138-86-3
Method/guideline	Measured
Melting Point	-97 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	CRC Handbook of Chemistry and Physics (1986) 67th edition, Robert C. Weast, editor, The Chemical Rubber Co Press, Inc. Boca Raton, Florida.

1.2 Boiling Point

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	176 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Fragrance Materials Association (FMA) Boiling point.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	178.6 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.

Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	CRC Handbook of Chemistry and Physics (1986) 67th edition, Robert C. Weast, editor, The Chemical Rubber Co Press, Inc. Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	175.5-176 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Merck Index (1996) 12th edition, Susan Budavari, editor, Merck & Co. Inc. Whitehouse Station, NJ.

Substance Name	*dl*-Limonene (dipentene)
CAS No.	138-86-3
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	175.5-176.5 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Merck Index (1996) 12th edition, Susan Budavari, editor, Merck & Co. Inc. Whitehouse Station, NJ.

Substance Name	*dl*-Limonene (dipentene)
CAS No.	138-86-3
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	178.6 °C
Pressure	760 mm Hg

Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	CRC Handbook of Chemistry and Physics (1986) 67th edition, Robert C. Weast, editor, The Chemical Rubber Co Press, Inc. Boca Raton, FL.

Substance Name	*dl*-Limonene (dipentene)
CAS No.	138-86-3
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	178 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Fragrance Materials Association (FMA) Boiling point.

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	185 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Fragrance Materials Association (FMA) Boiling point.

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	185.8 °C

Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	CRC Handbook of Chemistry and Physics (1986) 67th edition, Robert C. Weast, editor, The Chemical Rubber Co Press, Inc. Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	172 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Fragrance Materials Association (FMA) Boiling point.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	167.7 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	CRC Handbook of Chemistry and Physics (1986) 67th edition, Robert C. Weast, editor, The Chemical Rubber Co Press, Inc. Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Measured

GLP	Ambiguous
Boiling Point	44 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Merck Index (1996) 12th edition, Susan Budavari, editor, Merck & Co. Inc. Whitehouse Station, NJ.

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	165-168 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	CRC Handbook of Chemistry and Physics (1986) 67th edition, Robert C. Weast, editor, The Chemical Rubber Co Press, Inc. Boca Raton, FL.

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	Measured
GLP	Ambiguous
Boiling Point	158 °C
Pressure	760 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Fragrance Materials Association (FMA) Boiling point.

Substance Name	Terpenes & terpenoids, sweet orange oil
CAS No.	68647-72-3

Method/guideline	Measured
GLP	Ambiguous
Boiling Point	176 °C
Pressure	1013 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Givaudan-Roure (1991) Unpublished report to RIFM.

1.3 Vapor Pressure

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Measured
Vapor Pressure	1.43 mmHg (0.19 kPa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	IPCS (1998) Concise International Chemical Assessment Document No. 5 Limonene. World Health Organization, 5, Geneva.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Calculated
Vapor Pressure	1.2 mm Hg (0.16 kPa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Report values for vapor pressure.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Calculated/Mean of Antoine & Grain
Vapor Pressure	1.59 mmHg (0.19 kPa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI suite (2000) US Environmental Protection Agency.

Substance Name	*dl*-Limonene (dipentene)
CAS No.	138-86-3
Method/guideline	Calculated/Mean of Antoine & Grain
Vapor Pressure	1.63 mm Hg (0.22 kPa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI suite (2000) US Environmental Protection Agency.

Substance Name	*dl*-Limonene (dipentene)
CAS No.	138-86-3
Method/guideline	Calculated
Vapor Pressure	0.8 mm Hg (0.1 kPa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Report values for vapor pressure.

Substance Name	Terpinolene
CAS No.	586-62-9

Method/guideline	Calculated/Mean of Antoine & Grain
Vapor Pressure	1.44 mm Hg (0.19 kPa)
Temperature	25 ℃
Data Qualities Reliabilities	Reliability code 4. Not assignable
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI suite (2000) US Environmental Protection Agency.

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	Calculated
Vapor Pressure	0.5 mm Hg (0.07 kPa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Report values for vapor pressure.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Calculated
Vapor Pressure	1.5 mm Hg (0.2 kPa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Report values for vapor pressure.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Calculated/Mean of Antoine & Grain
Vapor Pressure	2.4 mm Hg (0.32 kPa)

Temperature	25 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI suite (2000) US Environmental Protection Agency.

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	Calculated/Mean of Antoine & Grain
Vapor Pressure	2.57 mm Hg (0.34 kPa)
Temperature	25 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI suite (2000) US Environmental Protection Agency.

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)
CAS No.	8008-57-9
Method/guideline	Calculated
Vapor Pressure	0.9 mm Hg (0.12 kPa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Report values for vapor pressure.

Substance Name	Terpenes & terpenoids, sweet orange oil
CAS No.	68647-72-3
Method/guideline	Calculated
Vapor Pressure	0.9 mm Hg (0.12 kPa)
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable

Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Report values for vapor pressure.

1.4 n-Octanol/Water Partition Coefficient

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Measured
GLP	Ambiguous
Log Pow	4.23
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	IPCS (1998) Concise International Chemical Assessment Document No. 5 Limonene. World Health Organization, 5, Geneva.

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	OECD Guideline No. 117
GLP	Yes
Year	1996
Log Pow	5.3
Temperature	30 °C
Remarks for Test Conditions	Reverse phase HPLC
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Givaudan Roure Inc. (1996a) Partition coefficient n-octanol/water of terpinolene. Private communication to RIFM.

Substance Name	Terpenes & terpenoids, sweet orange oil
CAS No.	68647-72-3

Method/guideline	OECD Guideline No. 117
GLP	Yes
Year	1996
Log Pow	5.3
Temperature	45 °C
Remarks for Test Conditions	Reverse phase HPLC
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Givaudan Roure Inc. (1996b) Partition coefficient n-octanol/water of orange peel. Private communication to RIFM.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Calculated
Log Pow	4.83
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Syracuse Research Corporation (SRC) Private communication to FMA.

Substance Name	*dl*-Limonene (dipentene)
CAS No.	138-86-3
Method/guideline	Calculated
Log Pow	4.83
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Syracuse Research Corporation (SRC) Private communication to FMA.

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	Calculated

Log Pow	4.88
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Syracuse Research Corporation (SRC) Private communication to FMA.

Substance Name	Myrcene
CAS No.	123-11-5
Method/guideline	Calculated
Log Pow	4.88
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Syracuse Research Corporation (SRC) Private communication to FMA.

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	Calculated
Log Pow	4.88
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Syracuse Research Corporation (SRC) Private communication to FMA.

1.5 Water Solubility

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Experimental
Value (mg/L) at Temperature	13.8 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.

References	WSKOWWIN EPI Suite (2000c) US Environmental Protection Agency (Massaldi, HA & King, CJ, 1973).

Substance Name	*dl*-Limonene (dipentene)
CAS No.	138-86-3
Method/guideline	Experimental
Value (mg/L) at Temperature	13.8 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	WSKOWWIN EPI Suite (2000c) US Environmental Protection Agency (Massaldi, HA & King, CJ, 1973).

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	Experimental
Value (mg/L) at Temperature	9.5 mg/L at 23 °C
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	WSKOWWIN EPI Suite (2000b) US Environmental Protection Agency (Li, J. and Perdue, EM, 1995).

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Experimental
Value (mg/L) at Temperature	5.6 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	WSKOWWIN EPI Suite (2000a) US Environmental Protection Agency (Chem Inspect Test Inst, 1992).

Substance Name	Terpenes & terpenoids, sweet orange oil
CAS No.	68647-72-3
Method/guideline	Experimental

Value (mg/L) at Temperature	30 mg/L at 20 °C
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Givaudan-Roure (1991) Unpublished report to RIFM.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Calculated
Value (mg/L) at Temperature	0.74 mg/L at 25 °C
Remarks for Test Conditions	Input parameters: Log Kow, 5.3; Melting Point, -74.35
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	WSKOWWIN EPI Suite (2000d) US Environmental Protection Agency.

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	Calculated
Value (mg/L) at Temperature	1.87mg/L at 25 °C
Remarks for Test Conditions	Input parameters: Log Kow, 4.88; Melting Point, -29.5
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	WSKOWWIN EPI Suite (2000d) US Environmental Protection Agency.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Calculated
Value (mg/L) at Temperature	1.87 mg/L at 25 °C
Remarks for Test Conditions	Input parameters: Log Kow, 4.88; Melting Point, -64.83
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated.
References	WSKOWWIN EPI Suite (2000d) US Environmental Protection Agency.

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	Calculated
Value (mg/L) at Temperature	1.87 mg/L at 25 °C
Remarks for Test Conditions	Input parameters: Log Kow, 4.88; Melting Point, -66.0
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	WSKOWWIN EPI Suite (2000d) US Environmental Protection Agency.

Substance Name	*dl*-Limonene (dipentene)
CAS No.	138-86-3
Method/guideline	Calculated
Value (mg/L) at Temperature	0.74 mg/L at 25 °C
Remarks for Test Conditions	Input parameters: Log Kow, 5.3; Melting Point, -97
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	WSKOWWIN EPI Suite (2000d) US Environmental Protection Agency.

2 Environmental Fate and Pathways

2.1 Photodegradation

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Calculated
Test Type	AOPWIN
Halflife t1/2	0.884 hours
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	AOPWIN EPI Suite (2000) US Environmental Protection Agency.

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	Calculated
Test Type	AOPWIN
Halflife t1/2	0.64 hours
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	AOPWIN EPI Suite (2000) US Environmental Protection Agency.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Calculated
Test Type	AOPWIN
Halflife t1/2	0.66 hours
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.

References	AOPWIN EPI Suite (2000) US Environmental Protection Agency.

2.2 Biodegradation

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	97% purity
Method	Not given
Test Type	Sealed vessel
GLP	Ambiguous
Year	1996
Contact Time	30 days
Innoculum	Unacclimated soil from coniferous forest
Remarks for Test Conditions	Limonene was tested in concentrations of 0.5-3 mg/liter, in sealed reactors. After 24 hours equilibration, inocula were added at 1%. Incubation took place in the dark at 23 degrees Celsius with continuous mixing. Duplicate gas and liquid samples were taken at regular intervals and analyzed for monoterpenes and CO2.
Degradation % After Time	0.044 mg/l h maximum degradation rate
Time required for 10% degradation	Less than 1 day
Results	Terpinolene was depleted below detectable limits within 1 day in acclimated soil and 8 days in unacclimated soil.
Classification	Readily biodegradable
Conclusion Remarks	The increase in biomass and headspace CO2 parallel the depletion of limonene, confirming that the disappearance of limonene was a result of biodegradation.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
Reference	Misra G., Pavlostathis S., Perdue E., Araujo R. (1996) Aerobic biodegradation of selected monoterpenes. Appl Microbiol Biotechnol 45: 831-838.

Substance Name	Terpinolene
CAS No.	586-62-9
Method	OECD Method 301B

Test Type	Sealed vessel carbon dioxide production test
GLP	Yes
Year	1996
Contact Time	28 days
Innoculum	Secondary effluent from an unacclimatized activated sludge plant
Remarks for Test Conditions	Nominal carbon concentrations of the test substances were used based on the calculated percentage carbon and assuming 100% purity of the named compound (1062 micrograms of carbon for terpinolene). The testing procedure followed OECD 301B.
Degradation % After Time	62.1% (95% C.I. 33.3-90.8) after 28 days
Time required for 10% degradation	Less than 3 days
Remarks Results	The air temperature during the 28 day test period was 19-24 degrees Celsius.
10 day window criteria	63.6%
Classification	PASS- readily and ultimately biodegradable
Conclusion Remarks	The test substance, terpinolene, was considered ultimately and readily biodegradable.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
Reference	Birch R.R. (1996) The ultimate biodegradability of terpinolene in the Sealed Vessel Test. Private Communication to FFHPVC.

Substance Name	Terpinolene
CAS No.	586-62-9
Remarks for Substance	82.2% pure by GC; almost colorless to pale yellow liquid; density 0.875 g/ml at 20 degrees Celsius
Method	OECD Method 302C
Test Type	Inherent Biodegradability: Manometric Respirometry Test
GLP	Yes
Year	1998
Contact Time	31 days
Innoculum	Fresh activated sludge
Remarks for Test Conditions	Followed OECD Method No. 302C. The reference substance used was sodium benzoate. The concentration of test substance used was 30 mg/l. The test temperature was 25 degrees Celsius.

Degradation % After Time	80% after 31 days (also 80% after 28 days)
Time required for 10% degradation	Less than 5 days
Results	The % degradation (mean of 2 identical flasks) after 5 days is 52%; 7 days is 54%; 14 days is 74%; 21 days is 80%; 28 days is 80% and 31 days is 80%.
10 day window criteria	Not given
Classification	Inherently biodegradable
Breakdown products (transient or stable)	Not given
Remarks fields for results	Averages of 2 identical flasks were used to determine the results. Degradation of sodium benzoate was 65% after 7 days and 86% after 14 days; the activity of the innoculum thus verified.
Conclusion Remarks	The test substance, terpinolene, underwent 80% biodegradation after 31 days under the test conditions and is considered inherently biodegradable.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Rudio J. (1998) Inherent biodegradability of terpinolene according to OECD Guideline No. 302C.Private communication to FFHPVC.

Substance Name	Terpinolene
CAS No.	586-62-9
Method	OECD Method 301F
Test Type	Ready Biodegradability: Manometric Respirometry Test
GLP	Yes
Year	1997
Contact Time	28 days
Innoculum	Fresh activated sludge
Remarks for Test Conditions	Followed OECD Method No. 301F. The reference substance used was sodium benzoate. The concentration of test substance used was 100 mg/l and the test temperature was 22 degrees Celsius.
Degradation % After Time	51% degradation after 28 days
Time required for 10% degradation	Less than 2 days
Results	The % degradation (mean of 2 identical flasks) after 2 days is 11%; 7 days is 31%; 12 days is 47%; 14 days is 49%; 21 days is 50% and 28 days is 51%.
10 day window criteria	47% at end of 10 day window (days 2 to 12)

Classification	Not readily biodegradable
Breakdown products (transient or stable)	Not given
Remarks fields for results	No toxic effects of terpinolene were observed on the microorganisms.
Conclusion Remarks	Terpinolene, which underwent 51% biodegradation after 28 days, should be regarded as not readily biodegradable under the conditions outlined for this test.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Rudio J. (1997) Ready biodegradability of pinene *alpha* according to OECD Guideline No. 301F. Private communication to FFHPVC.

Substance Name	Terpinolene
CAS No.	586-62-9
Remarks for Substance	97% purity
Method	Not given
Test Type	Sealed vessel
GLP	Ambiguous
Year	1996
Contact Time	30 days
Innoculum	Unacclimated soil from coniferous forest
Remarks for Test Conditions	Terpinolene was tested in concentrations of 0.5-3 mg/liter, in sealed reactors. After 24 hours equilibration, inocula were added at 1%. Incubation took place in the dark at 23 degrees Celsius with continuous mixing. Duplicate gas and liquid samples were taken at regular intervals and analyzed for monoterpenes and CO2.
Degradation % After Time	0.053 mg/l h maximum degradation rate
Time required for 10% degradation	Less than 1 day
Results	Limonene was depleted below detectable limits within 1 day in acclimated soil and 8 days in unacclimated soil.
Classification	Readily biodegradable
Conclusion Remarks	The increase in biomass and headspace CO2 parallel the depletion of terpinolene, confirming that the disappearance of terpinolene was a result of biodegradation.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.

Reference	Misra G., Pavlostathis S., Perdue E., Araujo R. (1996) Aerobic biodegradation of selected monoterpenes. Appl Microbiol Biotechnol 45: 831-838.

2.3 Fugacity

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Soil-Water Partition Coefficient
Absorption coefficient	731
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Sediment-Water Partition Coefficient
Absorption coefficient	1462
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Suspended Sediment-Water Partition Coefficient
Absorption coefficient	4570
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Air-Water Partition Coefficient
Absorption coefficient	1.05
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Fish-Water Partition Coefficient
Absorption coefficient	1858
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Aerosol-Air Partition Coefficient
Absorption coefficient	22700
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Air
Estimated Distribution and Media Concentration	93.8%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Water
Estimated Distribution and Media Concentration	0.18%
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Soil
Estimated Distribution and Media Concentration	5.87%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Sediment
Estimated Distribution and Media Concentration	0.13%
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Suspended Sediment
Estimated Distribution and Media Concentration	0.0041%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Fish
Estimated Distribution and Media Concentration	0.00033%
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Aerosol
Absorption coefficient	0.000043%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Air-Water Partition Coefficient
Absorption coefficient	0.57
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Sediment-Water Partition Coefficient
Absorption coefficient	1162
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Suspended Sediment-Water Partition Coefficient
Absorption coefficient	3630
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, calculated MP, water solubility
Media	Soil-Water Partition Coefficient
Absorption coefficient	581
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, VP, log Kow, MP, water solubility
Media	Fish-Water Partition Coefficient
Absorption coefficient	1477
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Aerosol-Air Partition Coefficient
Absorption coefficient	60600
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Air
Estimated Distribution and Media Concentration	91.2%
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Water
Estimated Distribution and Media Concentration	0.32%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Soil
Estimated Distribution and Media Concentration	8.32%
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Sediment
Estimated Distribution and Media Concentration	0.18%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Suspended Sediment
Estimated Distribution and Media Concentration	0.0058%
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Fish
Estimated Distribution and Media Concentration	0.00047%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Terpinolene
CAS No.	586-62-9
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Aerosol
Estimated Distribution and Media Concentration	0.000011%
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Air-Water Partition Coefficient
Absorption coefficient	2.64
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Soil-Water Partition Coefficient
Absorption coefficient	291
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Sediment-Water Partition Coefficient
Absorption coefficient	582
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Suspended Sediment-Water Partition Coefficient
absorption coefficient	1820
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Fish-Water Partition Coefficient
Absorption coefficient	740
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Aerosol-Air Partition Coefficient
Absorption coefficient	22300
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Air
Estimated Distribution and Media Concentration	98.9%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Water
Estimated Distribution and Media Concentration	0.075%
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Soil
Estimated Distribution and Media Concentration	0.098%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Sediment
Estimated Distribution and Media Concentration	0.022%
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Suspended Sediment
Estimated Distribution and Media Concentration	0.00068%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Fish
Estimated Distribution and Media Concentration	0.000056%
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

Substance Name	Myrcene
CAS No.	123-35-3
Model Conditions	25 °C, 100,000
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EQC V 2.11 Level I
Input Parameters	MW, log Kow, water solubility, VP & calculated MP
Media	Aerosol
Estimated Distribution and Media Concentration	0.000044%
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated. The data are obtained by a recognized fugacity calculation method.
References	Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC Press, Boca Raton, FL.

3 Ecotoxicity

3.1 Acute Toxicity to Fish

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	*d*-Limonene in neat form had purity of 99%, in toxicant saturated water of 85%, in exposure water 67%
Method/guideline	Flow-through. Calculated LC50 and EC50 using the Trimmed Spearman-Karber Method [Hamilton *et al*., 1977] and corrected average of the analyzed tank concentrations
Test Type	Experimental
GLP	Ambiguous
Year	1990
Species/Strain/Supplier	Fathead minnows/Fluorostat Corporation
Exposure Period	96 hour
Analytical monitoring	GC Analysis
Remarks for Test Conditions	96 hour LC50 and EC50 tests were performed with fathead minnows using *d*-limonene from two different suppliers. Test protocol was a continuous flow-through system. Tests were conducted in the electronic diluter using 0.2 L test chambers. A saturator flow rate of 21 ml/min resulted in exposure concentrations of 251 to 1890 ug/L.
Endpoint value	LC50 = 720 ug/L 95% C.L. (618-839 ug/L) and EC50=688 ug/L 95% C.L(606-782 ug/L).
Measured concentrations as mg/L	2.73 mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Broderius S. (1990) Toxicity of Eight Terpenes to Fathead Minnows, Daphnids, and Algae. U.S. EPA Environmental Research Laboratory-Duluth and ASCI Corporation.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	*d*-Limonene in neat form had purity of 99%, in toxicant saturated water of 85%, in exposure water 67%
Method/guideline	Flow-through. Calculated LC50 and EC50 using the Trimmed Spearman-Karber Method [Hamilton *et al*., 1977] and corrected average of the analyzed tank concentrations

Test Type	Experimental
GLP	Ambiguous
Year	1990
Species/Strain/Supplier	Fathead minnows/Aldrich Chemical
Exposure Period	96 hour
Analytical monitoring	GC Analysis
Remarks for Test Conditions	96 hour LC50 and EC50 tests were performed with fathead minnows using *d*-limonene from two different suppliers. Test protocol was a continuous flow-through system. Tests were conducted in the electronic diluter using 0.2 L test chambers. A saturator flow rate of 21 mls/min resulted in exposure concentrations of 178 to 1350 ug/L.
Endpoint value	LC50 = 702 ug/L 95% C.L. (619-796 ug/L) and EC50=702 ug/L 95% C.L(619-796 ug/L).
Measured concentrations as mg/L	1.81 mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Broderius S. (1990) Toxicity of Eight Terpenes to Fathead Minnows, Daphnids, and Algae. U.S. EPA Environmental Research Laboratory-Duluth and ASCI Corporation.

Substance Name	Terpinolene
CAS No.	586-62-9
Remarks for Substance	Terpinolene in neat form had purity of >99%, in toxicant saturated water of 75% purity, in exposure water 41% purity
Method/guideline	Calculated LC50 and EC50 using the Trimmed Spearman-Karber Method [Hamilton *et al*., 1977] and corrected average of the analyzed tank concentrations
Test Type	Experimental
GLP	Ambiguous
Year	1990
Species/Strain/Supplier	Fathead minnows/American Tokyo Kasei
Exposure Period	96 hour
Analytical monitoring	GC Analysis
Remarks for Test Conditions	96 hour LC50 and EC50 tests were performed with fathead minnows using terpinolene. Test protocol was a continuous flow-through system. Tests were conducted in the electronic diluter using 0.2 L test chambers. A saturator flow rate of 21 mL/min resulted in exposure concentrations of 279 to 3020 ug/L.

Endpoint value	LC50 = 720 ug/L 95% C.L. (618-839 ug/L) and EC50=688 ug/L 95% C.L(606-782 ug/L).
Measured concentrations as mg/L	3.38 mg/L
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Broderius S. (1990) Toxicity of Eight Terpenes to Fathead Minnows, Daphnids, and Algae. U.S. EPA Environmental Research Laboratory-Duluth and ASCI Corporation.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Fish
Exposure Period	96 hour
Remarks for Test Conditions	Input parameters: Melting point, -74.35, Water solubility - 13.8 mg/L, Log Kow - 5.3
Endpoint value	LC50 = 0.221 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

Substance Name	138-86-3
CAS No.	*dl*-Limonene (dipentene)
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Fish
Exposure Period	96 hour
Remarks for Test Conditions	Input parameters: Melting point, -74.35, Water solubility - 13.8 mg/L, Log Kow - 5.3
Endpoint value	LC50 = 0.221 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.

Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Fish
Exposure Period	96 hour
Remarks for Test Conditions	Input parameters: Melting point, -29.50, Water solubility - 9.5 mg/L, Log Kow - 4.88
Endpoint value	LC50 = 0.198 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Fish
Exposure Period	96 hour
Remarks for Test Conditions	Input parameters: Melting point, -64.83, Water solubility - 5.6mg/L, Log Kow - 4.8
Endpoint value	LC50 = 0.198 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	ECOSAR

Test Type	Calculated
Species/Strain/Supplier	Fish
Exposure Period	96 hour
Remarks for Test Conditions	Input parameters: Melting point, -66.11, Log Kow - 4.88
Endpoint value	LC50 = 0.201 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

3.2 Acute Toxicity to Aquatic Invertebrates

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	*d*-Limonene in neat form had purity of 99%, in toxicant saturated water of 85%, in exposure water 67%
Method/guideline	Flow-through. Calculated LC50 and EC50 using the Trimmed Spearman-Karber Method [Hamilton *et al*., 1977] and corrected average of the analyzed tank concentrations
Test Type	Experimental
GLP	No
Species/Strain/Supplier	*Daphnia Magna*
Analytical procedures	GC Analysis
Test Details	96 hours
Remarks for Test Conditions	96 hour LC50 and EC50 tests were performed with daphnia magna from two different suppliers. Test protocol was a continuous flow-through system. Tests were conducted in the electronic diluter using 0.2 L test chambers. A saturator flow rate of 21 mL/min resulted in exposure concentrations of 251 to 1890 ug/L.
Measured concentrations as mg/L	2.73 mg/L
EC50, EL50, LC0, at 24,48 hours	96 hr LC50 = .577 mg/L 95% C.L. (496-672 ug/L) and EC50=421 ug/L (no reliable confidence limits)
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Data Reliability Remarks	Code 1. Guideline study.
Reference	U.S. EPA Environmental Research Laboratory-Duluth and ASCI Corporation.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	*d*-Limonene in neat form had purity of 99%, in toxicant saturated water of 85%, in exposure water 67%
Method/guideline	Calculated LC50 and EC50 using the Trimmed Spearman-Karber Method [Hamilton *et al*., 1977] and corrected average of the analyzed tank concentrations
Test Type	Experimental
GLP	No
Species/Strain/Supplier	*Daphnia Magna*
Analytical procedures	GC Analysis
Test Details	96 hours
Remarks for Test Conditions	96 hour LC50 and EC50 tests were performed with daphnia magna using *d*-limonene from two different suppliers. Test protocol was a continuous flow-through system. Tests were conducted in the electronic diluter using 0.2 L test chambers. A saturator flow rate of 21 mL/min resulted in exposure concentrations of 178 to 1350 ug/L.
Measured concentrations as mg/L	1.81 mg/L
EC50, EL50, LC0, at 24,48 hours	96 hr LC50 = .924 mg/L (no reliable confidence limits)
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Data Reliability Remarks	Code 1. Guideline study.
Reference	U.S. EPA Environmental Research Laboratory-Duluth and ASCI Corporation.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	97% purity
Method/guideline	Static. EC50
Test Type	Experimental
GLP	Ambiguous
Year	1987
Species/Strain/Supplier	*Daphnia pulex*
Analytical procedures	GC Analysis

Test Details	48 hours
Remarks for Test Conditions	The 48 hour static acute tests with Daphnia pulex were conducted without feeding the organisms. Tests included a water or solvent control and five concentrations of toxicant dissolved in water or toxicant. Tests chambers contained at least 10 neonates in 150 ml of water. Two replicates were performed.
EC50, EL50, LC0, at 24,48 hours	EC50 = 69.6 mg/L at 48 hour
Data Qualities Reliabilities	Reliability code 3. Not reliable.
Data Reliability Remarks	Code 3. Test concentration exceeds the limit of solubility of *d*-limonene.
Reference	Passino D.R., and Smith S. (1987) Acute bioassays and hazard evaluation of representative contaminants detected in great lakes fish. Environmental Toxicology and Chemistry, 6, 901.

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	Flow-through. Calculated LC50 and EC50 using the Trimmed Spearman-Karber Method [Hamilton *et al*., 1977] and corrected average of the analyzed tank concentrations
Test Type	Experimental
GLP	No
Species/Strain/Supplier	*Daphnia Magna*
Analytical procedures	GC Analysis
Test Details	96 hours
Remarks for Test Conditions	96 hour LC50 and EC50 tests were performed with daphnia magna using terpinolene. Test protocol was a continuous flow-through system. Tests were conducted in the electronic diluter using 0.2 L test chambers. A saturator flow rate of 21 mL/min resulted in exposure concentrations of 279 to 3020 ug/L.
Measured concentrations as mg/L	3.38 mg/L
EC50, EL50, LC0, at 24,48 hours	96 hr LC50 = 2.55 mg/L (no reliable confidence limits) and EC50=1380 ug/L (no reliable confidence limits)
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Data Reliability Remarks	Code 1. Guideline study.
Reference	U.S. EPA Environmental Research Laboratory-Duluth and ASCI Corporation.

Substance Name	Myrcene
CAS No.	123-35-3

Method/guideline	EPA Committee on Methods for Toxicity Tests with Aquatic Organisms (without replicate concentrations)
Test Type	Experimental
GLP	Ambiguous
Year	1986
Species/Strain/Supplier	*Daphnia Magna*
Test Details	48 hours
Remarks for Test Conditions	200 ml of the test solution and 10 Daphnia were used. Dissolved oxygen and pH were determined initially and at 48 hour. Mortalities were recorded at 24 and 48 hour.
EC50, EL50, LC0, at 24,48 hours	LC50 = 31 mg/L at 48 hour
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Data Reliability Remarks	Code 2. Basic data given: comparable to guidelines/standards.
Reference	Waggy G.L. and Blessing R.L. (1986) Private communication.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Brine shrimp lethality test [Meyer, 1982]
Test Type	Experimental
GLP	Ambiguous
Year	1998
Species/Strain/Supplier	Brine shrimp
Analytical procedures	GC Analysis
Test Details	24 hours
Remarks for Test Conditions	The essential oil of Apium graveolens (celery) was analyzed by GC-MS. Components were isolated using dry column chromatography and low pressure liquid chromatography to isolate the hydrocarbon component and individual hydrocarbon compounds, respectively. The brine shrimp lethality test was performed using the crude volatile oil, the hydrocarbon and oxygenated fractions, and the isolated compounds. The results given here are for the respective isolated compounds as listed under Substance Name. Brine shrimp eggs were hatched in seawater and used after 48 hrs. Ten shrimps were added to three vials for each of the following doses (2, 50 and 200 ppm). The vials were prepared by thoroughly mixing with the allotted amount of artificial seawater to achieve the correct concentration. The number of

	deaths out of 30 shrimps per dose were recorded after 24 hrs. LC50 values and corresponding 95% confidence intervals were determined. Compounds with LC50 values over 200 ppm were retested at 1000 ppm. LC50 values greater than 200 ppm were considered inactive for pure compounds.
EC50, EL50, LC0, at 24,48 hours	LC50 = 39.2 mg/L
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Data Reliability Remarks	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
Reference	Saleh M.M., Hashem F.A., Glombitza K.A. (1998) Cytotoxicity and invitro effects on human cancer cell lines of volatiles of Apium graveolens var. filicinum. Pharmaceutical Pharmacological Letters, 8(2), 97-99.

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	Brine shrimp lethality test [Meyer, 1982]
Test Type	Experimental
GLP	Ambiguous
Year	1998
Species/Strain/Supplier	Brine shrimp
Analytical procedures	GC Analysis
Test Details	24 hours
Remarks for Test Conditions	The essential oil of Apium graveolens (celery) was analyzed by GC-MS. Components were isolated using dry column chromatography and low pressure liquid chromatography to isolate the hydrocarbon component and individual hydrocarbon compounds, respectively. The brine shrimp lethality test was performed using the crude volatile oil, the hydrocarbon and oxygenated fractions, and the isolated compounds. The results given here are for the respective isolated compounds as listed under Substance Name. Brine shrimp eggs were hatched in seawater and used after 48 hrs. Ten shrimps were added to three vials for each of the following doses (2, 50 and 200 ppm). The vials were prepared by thoroughly mixing with the allotted amount of artificial seawater to achieve the correct concentration. The number of deaths out of 30 shrimps per dose were recorded after 24 hrs. LC50 values and corresponding 95% confidence intervals were determined. Compounds with LC50 values over 200 ppm were retested at 1000 ppm. LC50 values greater than 200 ppm were considered inactive for pure compounds.
EC50, EL50, LC0, at 24,48 hours	LC50 =104.1 mg/L

Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Data Reliability Remarks	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
Reference	Saleh M.M., Hashem F.A., Glombitza K.A. (1998) Cytotoxicity and invitro effects on human cancer cell lines of volatiles of Apium graveolens var. filicinum. Pharmaceutical Pharmacological Letters, 8(2), 97-99.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	*Daphnia Magna*
Test Details	48 hours
EC50, EL50, LC0, at 24,48 hours	LC50 = 0.496 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	*Daphnia Magna*
Test Details	48 hours
EC50, EL50, LC0, at 24,48 hours	LC50 = 0.612 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

Substance Name	Myrcene
CAS No.	123-35-3

Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	*Daphnia Magna*
Test Details	48 hours
EC50, EL50, LC0, at 24,48 hours	LC50 = 1.147 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	*Daphnia Magna*
Test Details	48 hours
EC50, EL50, LC0, at 24,48 hours	LC50 = 0.263 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

3.3 Acute Toxicity to Aquatic Plants

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	*d*-Limonene in neat form had purity of 99%, in toxicant saturated water of 85%, in exposure water 67%
Method/guideline	ASTM methods (ASTM, 1988)
Test Type	Static 96 hour toxicity test with micro algae
GLP	Ambiguous
Year	1990

Species/Strain/Supplier	Green algae/*Selenastrum capricornutum*
Exposure Period	96 hour
Analytical monitoring	GC analysis
Remarks for Test Conditions	Test conditions followed ASTM methods. Changes were made to help reduce any problems associated with volatility. GC analysis was performed at 0, 24, and 96 hours. The 96 hour sample was performed only if the chemical was detected at the 24 hour sampling. Test cell concentrations were approximately 10000 cells/mL. Coulter counters and electronic particle counter were used to count cells and determine mean cell volume. The IC50 (the concentration at which 50% growth inhibition) was calculated using a linear interpolation program.
Nominal concentrations as mg/L	Not given
Measured concentrations as mg/L	1.81 mg/L
Endpoint value	No significant inhibition
Conclusion Remarks	No significant inhibition.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Broderius S. (1990) Toxicity of Eight Terpenes to Fathead Minnows, Daphnids, and Algae. U.S. EPA Environmental Research Laboratory-Duluth and ASCI Corporation.

Substance Name	Terpinolene
CAS No.	586-62-9
Remarks for Substance	Terpinolene in neat form had purity of >99%, in toxicant saturated water of 75% purity, in exposure water 41% purity
Method/guideline	ASTM methods (ASTM, 1988)
Test Type	Static 96 hour toxicity test with micro algae
GLP	Ambiguous
Year	1990
Species/Strain/Supplier	Green algae/*Selenastrum capricornutum*
Exposure Period	96 hour
Analytical monitoring	GC analysis
Remarks for Test Conditions	Test conditions followed ASTM methods. Changes were made to help reduce any problems associated with volatility. GC analysis was performed at 0, 24, and 96 hours. The 96 hour sample was performed only if the chemical was detected at the 24 hour sampling. Test cell concentrations were approximately 10000 cells/mL. Coulter counters and electronic particle counter

	were used to count cells and determine mean cell volume. The IC50 (the concentration at which 50% growth inhibition) was calculated using a linear interpolation program.
Nominal concentrations as mg/L	Not given
Measured concentrations as mg/L	3.38 mg/L
Endpoint value	No significant inhibition
Conclusion Remarks	No significant inhibition.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	Broderius S. (1990) Toxicity of Eight Terpenes to Fathead Minnows, Daphnids, and Algae. U.S. EPA Environmental Research Laboratory-Duluth and ASCI Corporation.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Green algae
Exposure Period	96 hours
Endpoint value	EC50 = 0.360 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Green algae
Exposure Period	96 hours
Endpoint value	EC50 = 0.813 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Green algae
Exposure Period	96 hours
Endpoint value	EC50 = 0.441 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	ECOSAR
Test Type	Calculated
Species/Strain/Supplier	Green algae
Exposure Period	96 hours
Endpoint value	EC50 = 0.194 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

4 Human Health Toxicity

4.1 Acute Toxicity

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Litchfield and Wilcoxon, 1949
Test Type	Oral LD 50
GLP	No
Year	1975
Species/strain	Rat/Wistar
Sex	Male and Female
# of animals per sex per dose	10 male 10 female
Vehicle	Arabic gum/water
Route of Administration	Oral
Remarks for Test Conditions	Ten male and ten female rats per group were used.
Value LD50 or LC50 with confidence limits	Male LD50 = 4400 (3400-5900) mg/kg bw (95% confidence limit) and female LD50= 5.2 (3.9-7.0) g/kg bw (95% confidence limit)
Number of deaths at each dose level	1500 mg/kg NOE in males. Not tested in females. 1900 mg/kg LTL 30% mortality in males. No observed effects in females. 2500 mg/kg LTL 30% mortality in males. 20% mortality in females. 3.3 g/kg LTL 40% mortality in males. 50% mortality in females. 4.3 g/kg LTL 60% mortality in males. 40% mortality in females. 4.4 g/kg LD50 male rats. 95% Confidence limits (3.4-5.9). 5.2 g/kg LD50 female rats. 95% Confidence limits (3.9-7.0). 5.6 g/kg LTL 40% mortality in males. 50% mortality in females. 7.3 g/kg LTL 90% mortality in males. 60% mortality in females. 9.4 g/kg LTL 70% mortality in males. 60% mortality in females. 12.2 g/kg LTL 80% mortality in males. 90% mortality in females. 15.9 g/kg LTL 90% mortality in males. 100% mortality in females.
Remarks for Results	The male LD50 = 4400 (3400-5900) mg/kg bw (95% confidence limits) and female LD50= 5200 (3900-7000) mg/kg bw (95% confidence limits).
Conclusion remarks	The male LD50 = 4400 (3400-5900) mg/kg bw (95% confidence limits) and female LD50= 5200 (3900-7000) mg/kg bw (95% confidence limits).
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.

References	Tsuji M., Y.Fujisaki, Y.Arikawa, S.Masuda, S.Kinoshita, A.Okubo, K.Noda, H.Ide and Y.Iwanaga (1975a) Studies on *d*-limonene as a gallstone solubilizer: Acute and sub-acute toxicities. Journal Oyo Yakuri, 9, 387-401.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Litchfield and Wilcoxon, 1949
Test Type	Oral LD 50
GLP	No
Year	1975
Species/strain	Mouse
Sex	Male and Female
# of animals per sex per dose	10 male 10 female
Vehicle	Arabic gum/water
Route of Administration	Oral
Remarks for Test Conditions	Ten male and ten female mice per group were used.
Value LD50 or LC50 with confidence limits	Male LD50 = 5600 (4800-6500) mg/kg bw (95% confidence limit) and female LD50 = 6600 (5500-7900) mg/kg bw (95% confidence limit)
Number of deaths at each dose level	3.0 g/kg NOE in male mice. 3.5 g/kg LTL 10% mortality in male mice. No observable effects in female mice. 4.3 g/kg LTL 20% mortality in female mice. No observable effects in male mice. 5.3 g/kg LTL 20% mortality in female mice. 30% mortality in male mice. 5.6 g/kg LD50 male mice. 95% confidence limits (4.8-6.5) 6.6 g/kg LD50 female mice. 95% confidence limits (5.5-7.9). 7.0 g/kg LTL 30% mortality in female mice. 60% mortality in male mice. 7.5 g/kg LTL 60% mortality in female mice. 90% mortality in male mice. 8.3 g/kg LTL 90% mortality in female mice. 100% mortality in male mice. 10.0 g/kg LTL 100% mortality in female mice.
Remarks for Results	The male LD50 = 5600 (4800-6500) mg/kg bw (95% confidence limits) and female LD50 = 6600 (5500-7900) mg/kg bw (95% confidence limits).
Conclusion remarks	Male LD50 = 4400 (3400-5900) mg/kg bw (95% confidence limit) and female LD50 = 5200 (3900-7000) mg/kg bw (95% confidence limits).
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Tsuji M., Y.Fujisaki, Y.Arikawa, S.Masuda, S.Kinoshita, A.Okubo, K.Noda, H.Ide and Y.Iwanaga (1975a) Studies on *d*-limonene as a gallstone solubilizer: Acute and sub-acute

toxicities. Journal Oyo Yakuri, 9, 387-401.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Not given
Test Type	Oral LD 50
GLP	No
Year	1972
Species/strain	Rat/Wistar
Sex	Male
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Oral
Remarks for Test Conditions	Ten male albino Wistar rats per group were used. Animals were fasted for a minimum of 16 hours prior to administration of the test material. Animals weighed 200-250 grams. Following dosing the animals received food and water ad libitum. Observations for mortality were made at 1 and 6 hours after dosing and daily thereafter for 14 days. Toxic effects were also observed. Gross necropsies were performed on all survivors.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Number of deaths at each dose level	None
Remarks for Results	Animals experienced lethargy. No deaths occurred. Oral LD50 greater than 5000 mg/kg.
Conclusion remarks	Oral LD50 greater than 5000 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1972c) Acute oral toxicity of limonene in rats. Unpublished report.

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	Not given
Test Type	Oral LD 50

GLP	No
Year	1975
Species/strain	Rat
Sex	Not reported
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Oral
Value LD50 or LC50 with confidence limits	4.39 ml/kg 95% Confidence Limits (3.75-5.14 ml/kg)
Number of deaths at each dose level	3.0 ml/kg 0 deaths3.5 ml/kg 1 death4.0 ml/kg 5 deaths 5.0 ml/kg 6 deaths
Remarks for Results	The oral LD50 was calculated to be 4.39 ml/kg bw with 95% C.L. (3.75-5.14 ml/kg).
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Levenstein I. (1975) Oral LD 50 of terpinolene in rats. Unpublished report to RIFM.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Not given
Test Type	Oral LD 50
GLP	Ambiguous
Year	1990
Species/strain	Rat/Wistar
Sex	Male and Female
# of animals per sex per dose	1 male 1 female except three highest doses
Vehicle	Corn oil
Route of Administration	Oral-Gavage
Remarks for Test Conditions	Two animals (1 male and 1 female) were used per dose with the exception of the three highest doses where 2 animals of each sex were used (see number of deaths at each dose level). Animals were fasted overnight prior to test administration. Animals were observed for 14 days following administration. Necropsies were performed on all animals.
Value LD50 or LC50 with confidence limits	Greater than 11.39 g/kg bw

Number of deaths at each dose level	0 g/kg bw Male 0/1 Female 0/1 0.67 g/kg bw Undefined results 1.00 g/kg bw Male 0/1Female 0/1 1.5 g/kg bw Male 0/1 Female 0/1, 2.25 g/kg bw Male 0/1 Female 0/1 3.25 g/kg bw Male 0/1 Female 0/1 5.06 g/kg bw Male 0/2 Female 0/2 7.59 g/kg bw Male 0/2 Female 0/2 11.39 g/kg bw Male 0/2 Female 0/2
Remarks for Results	Animals at the three highest dose levels experienced palpebral ptosis, hypoactivity, and ataxia.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Paumgartten F.J.R. (1990) Single Dose Toxicity of *beta*-myrcene; a natural analgesic substance. Brazilian J Med Biol Res 23; 873-877.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Not given
Test Type	Oral LD 50
GLP	Ambiguous
Year	1990
Species/strain	Mouse/Albino Swiss
Sex	Male and Female
# of animals per sex per dose	1 male 1 female with the exception of 2 doses
Vehicle	Corn oil
Route of Administration	Oral-Gavage
Remarks for Test Conditions	Two animals (1 male and 1 female) were used per dose with the exception of two doses where 3 animals of each sex were used (see number of deaths at each dose level). Animals were fasted overnight prior to test administration. Animals were observed for 14 days following administration. Necropsies were performed on all animals.
Value LD50 or LC50 with confidence limits	5060 mg/kg bw
Number of deaths at each dose level	0 g/kg bw Male 0/1 Female 0/1 0.67 g/kg bw Undefined results 1.00 g/kg bw Male 0/1 Female 0/1 1.5 g/kg bw Male 0/1 Female 0/1 2.25 g/kg bw Male 0/1 Female 0/1 3.25 g/kg bw Male 0/1 Female 0/1

	5.06 g/kg bw Male 2/3 Female 3/3 7.59 g/kg bw Male 3/3 Female 2/3 11.39 g/kg bw Male 1/1 Female 1/1
Remarks for Results	Animals at the three highest dose levels experienced palpebral ptosis, hypoactivity, and ataxia.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Paumgartten F.J.R. (1990) Single Dose Toxicity of *beta*-myrcene; a natural analgesic substance. Brazilian J Med Biol Res 23; 873-877.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Not given
Test Type	Oral LD 50
GLP	No
Year	1972
Species/strain	Rat/Wistar
Sex	Male
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Oral
Remarks for Test Conditions	Ten male albino Wistar rats per group were used. Animals were fasted for a minimum of 16 hours prior to administration of the test material. Animals weighed 200-250 grams. Following dosing the animals received food and water ad libitum. Observations for mortality were made at 1 and 6 hours after dosing and daily thereafter for 14 days. Toxic effects were also observed. Gross necropsies were performed on all survivors.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Number of deaths at each dose level	1/10 deaths. Death occurred overnight following administration. Clinical signs included lethargy and urinary incontinence.

Remarks for Results	Animals experienced lethargy urinary incontinence. One deaths occurred. Oral LD50 greater than 5000 mg/kg.
Conclusion remarks	Oral LD50 greater than 5000 mg/kg.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1972d) Acute oral toxicity of myrcene in rats. Unpublished report.

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	Litchfield and Wilcoxon, 1949
Remarks for Substance	Clear liquid
Test Type	Oral LD 50
GLP	No
Year	1980
Species/strain	Rat/Wistar
Sex	Male
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Oral
Remarks for Test Conditions	The rats were observed for 3-4 hours after dosing and once daily for 14 days. Mortality, toxicology, and pharmacological effects were recorded.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Number of deaths at each dose level	1 death at 5000 mg/kg bw
Remarks for Results	Lethargy and piloerection were noted 3-4 hours post dose. Most animals were generally healthy thereafter.
Conclusion remarks	Oral LD50 greater than 5000 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1980b) Acute oral toxicity of dihydromyrcene in rats. Unpublished report.

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)

CAS No.	8008-57-9
Method/guideline	Not given
Test Type	Oral LD 50
GLP	No
Year	1973
Species/strain	Rat/Wistar
Sex	Male
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Oral
Remarks for Test Conditions	The rats were observed for 3-4 hours after dosing and once daily for 14 days. Mortality, toxicology, and pharmacological effects were recorded.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Remarks for Results	The oral LD50 was determined to be greater than 5000 mg/kg bw.
Conclusion remarks	Oral LD50 >5000 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1973b) Acute oral toxicity of orange oil in rats. Unpublished report.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Not given
Remarks for Substance	Clear liquid
Test Type	Dermal LD50
GLP	No
Year	1972
Species/strain	Rabbit/New Zealand White
Sex	Not reported
# of animals per sex per dose	10

Vehicle	None
Route of Administration	Dermal
Remarks for Test Conditions	A single 24 hour application of limonene (5 g/kg) was applied to the clipped abraded abdominal skin of 10 rabbits weighing from 1.9 to 2.4 kg. Observations for mortality and toxic effects were made for seven days following exposure. Gross necropsies were performed on all animals at study termination.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Number of deaths at each dose level	None
Remarks for Results	No animals died during the study. Additionally, there was no evidence of toxicity resulting from application of the material.
Conclusion remarks	Oral LD50 greater than 5000 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1972a) Acute dermal toxicity of limonene in rabbits. Unpublished report.

Substance Name	Dihydromyrcene
CAS No.	2436-90-0
Method/guideline	Litchfield and Wilcoxon, 1949
Remarks for Substance	Clear liquid
Test Type	Dermal LD50
GLP	No
Year	1980
Species/strain	Rabbit/New Zealand White
Sex	Male
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Dermal
Remarks for Test Conditions	A single 24 hour application of dihydromyrcene (5 g/kg) was applied to the clipped abraded abdominal skin of 10 rabbits. Observations for mortality and toxic effects were made for 14 days following exposure. All animals were examined for gross pathology.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Number of deaths at each dose level	1 death at 5000 mg/kg bw

Remarks for Results	Toxic signs noted infrequently included lethargy and diarrhea. Internal organs of surviving animals were normal following superficial examination. One animal showed signs of cardiac and respiratory abnormalities. Most also had skin abnormalities.
Conclusion remarks	Dermal LD50 >5000 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1980b) Acute dermal toxicity of dihydromyrcene in rabbits. Unpublished report.

Substance Name	Terpinolene
CAS No.	586-62-9
Method/guideline	Not given
Test Type	Dermal LD50
GLP	No
Year	1975
Species/strain	Rat
Sex	Not reported
# of animals per sex per dose	4
Vehicle	None
Route of Administration	Dermal
Value LD50 or LC50 with confidence limits	Greater than 5 ml/kg bw
Number of deaths at each dose level	0 deaths
Remarks for Results	The dermal LD50 was determined to be greater than 5 ml/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Levenstein I. (1975) Dermal LD 50 of terpinolene in rats. Unpublished report to RIFM.

Substance Name	Myrcene
CAS No.	123-35-3
Method/guideline	Not given
Test Type	Dermal LD50

GLP	No
Year	1972
Species/strain	Rabbit/New Zealand White
Sex	Not reported
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Dermal
Remarks for Test Conditions	A single 24 hour application of terpinolene (5 g/kg) was applied to the clipped abraded abdominal skin of 10 rabbits weighing from 1.9 to 2.4 kg. Observations for mortality and toxic effects were made for seven days following exposure. Gross necropsies were performed on all animals at study termination.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Number of deaths at each dose level	No deaths during the course of the study. No evidence of toxicity from percutaneous absorption of the test substance. Erythema and edema were reported during the first few days of observation, but cleared by the study termination.
Remarks for Results	The dermal LD50 was determined to be greater than 5000 mg/kg bw.
Conclusion remarks	Dermal LD50 greater than 5000 mg/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1972b) Acute dermal toxicity of myrcene in rabbits. Unpublished report.

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)
CAS No.	123-35-3
Method/guideline	Not given
Test Type	Dermal LD50
GLP	No
Year	1973
Species/strain	Rabbit/New Zealand White
Sex	Male
# of animals per sex per dose	10
Vehicle	None

Route of Administration	Dermal
Remarks for Test Conditions	A single 24 hour application of terpinolene (5 g/kg) was applied to the clipped abraded abdominal skin of 10 rabbits weighing from 1.9 to 2.4 kg. Observations for mortality and toxic effects were made for seven days following exposure. Gross necropsies were performed on all animals at study termination.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Remarks for Results	The dermal LD50 was determined to be greater than 5000 mg/kg bw.
Conclusion remarks	Dermal LD50 greater than 5.0 g/kg bw
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Moreno O. (1973a) Acute dermal toxicity of orange oil in rabbits. Unpublished report.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	Purity undetermined
Method/guideline	Not given
Test Type	Inhalation ED25
GLP	Not reported
Year	1977
Species/strain	Mouse
Sex	Female
# of animals per sex per dose	5
Vehicle	None
Route of Administration	Inhalation
Remarks for Test Conditions	The respiratory irritation potential of fragrance raw materials was assessed in CF-1 females by recording respiratory rate using a whole body plethysmograph. Mice were exposed to test materials for 1 min using a nebulizer for aerosolization in a 2600 ml chamber. Materials shown to be sensory irritants were further tested in mice cannulated via the trachea & compared to an intact mouse breathing through its nose. Comparisons made were between the pre-exposure & exposure rate values for each material at each dose level. Materials were of undetermined purity. Respiratory tract, via nose, mil*d*-moderate resp depression; ED25 = 570 ug/l; no effects when inhaled through tracheal cannula. (Troy, 1977) 9011

Value LD50 or LC50 with confidence limits	No ED25 determined.
Number of deaths at each dose level	None
Remarks for Results	Slight respiratory depression. Lower tract exposures not performed. No dose-response relationship.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Troy W.R. (1977) Doctoral Dissertation: The comparative respiratory irritation potential of fourteen fragrance raw materials. Unpublished report to RIFM.

4.2 Genetic Toxicity

4.2.1 In vitro Genotoxicity

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Ames test
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	No
Year	1980
Species/Strain	*Salmonella typhimurium* TA 98, TA 100, TA 1535 and TA 1537
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	0.03, 0.3, 3 and 30 micromol/plate for TA98 and TA100; 3 micromol/plate for the remaining strains
Remarks for Test Conditions	The solvent used was ethanol. Only one replicate was performed for the substances, which tested negative.
Results	No mutagenic effects.
Cytotoxic concentration	Greater than 3 micromoles/plate
Genotoxic Effects	None
Appropriate statistical evaluations	None given
Remarks for results	Limonene was inactive in *Salmonella* strains TA 1535, TA 1537, TA 98 & TA 100 both in the presence and absence of metabolic activation system.
Conclusion Remarks	No evidence of mutagenic activity.

Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Florin I., Rutberg L., Curvall M., and Enzell C.R. (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames test. Toxicology, 18 pages 219-232.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Ames test
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	No
Year	1989
Species/Strain	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, and TA1538
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	150000 micrograms/plate
Remarks for Test Conditions	After two days incubation at 37 °C, revertant colonies were counted.
Results	No mutagenic effects.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations	None given
Remarks for results	Limonene was inactive in Salmonella strains TA 1535, TA 1537, TA 1538, TA 98 & TA 100 both in the presence and absence of metabolic activation system.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Heck, J. D., Vollmuth, T. A., Cifone, M. A., Jagannath, D. R., Myhr B., and R.D. Curren (1989). An evaluation of food flavoring ingredients in a genetic toxicity screening battery The Toxicologist, 9(1), 257.

Substance Name	*d*-Limonene
CAS No.	5989-27-5

Remarks for Substance	Solvents used included DMSO and/or ethanol.
Method/guideline	Ames
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1993
Species/Strain	*Salmonella typhimurium* TA102
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats.
Doses/Concentration	Up to 5000 micrograms per plate
Statistical Methods	Not given
Remarks for Test Conditions	Limonene was tested in two independent experiments using five doses and three plates per dose. Solvents used for diluting the compounds were DMSO, ethanol and water. All compounds were tested up to 5000 micrograms per plate if possible unless limited by cytotoxicity or precipitation.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	Negative
Appropriate statistical evaluations	Not given
Remarks for results	Limonene was inactive in *Salmonella* strains TA 102 in the presence and absence of metabolic activation system.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Muller W. (1993) Evaluation of mutagenicity testing with Salmonella typhimurium TA102 in three different laboratories. Environmental Health Perspectives Supplemnts. 101, 33-36.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	99.7% analyzed purity
Method/guideline	Ames test
Test Type	Ames reverse mutation
System of Testing	Bacterial

GLP	Ambiguous
Year	1983
Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA98, TA100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats
Doses/concentration levels	0.3-3333 micrograms/plate
Statistical Methods	Model used presented in Margolin *et al*., 1981
Remarks for Test Conditions	At least five doses of the test chemical, in addition to solvent and positive controls, were tested on each strain in the presence of S9 mix or buffer. Three plates were used and the experiment was repeated no less than 1 week after completion of the initial test.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations	Yes
Remarks for results	Limonene was inactive in *Salmonella* strains TA 1535, TA1537, TA98, and TA100 in the presence and absence of metabolic activation system.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Haworth, S., Lawlor T., Mortelmans K., Speck W., and Zeiger E. (1983) Salmonella Mutagenicity Test Results for 250 Chemicals. Environmental Mutagenesis Supplement 1, 3-142.

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)
CAS No.	8008-57-9
Method/guideline	Ames test
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	No
Year	1989
Species/Strain	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, and TA1538
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats

Doses/Concentration	5000 micrograms/plate
Remarks for Test Conditions	After two days incubation at 37 °C, revertant colonies were counted.
Results	No mutagenic effects.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations	None given
Remarks for results	Orange oil was inactive in *Salmonella* strains TA 1535, TA 1537, TA 1538, TA 98 & TA 100 both in the presence and absence of metabolic activation system.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Heck, J. D., Vollmuth, T. A., Cifone, M. A., Jagannath, D. R., Myhr B., and R.D. Curren (1989). An evaluation of food flavoring ingredients in a genetic toxicity screening battery The Toxicologist, 9(1), 257.

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)
CAS No.	8008-57-9
Method/guideline	Ames test
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1990
Species/Strain	*Salmonella typhimurium* TA 98 and TA 100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	100 microliters/plate
Remarks for Test Conditions	S-9 level used was 50 microliters/plate.
Results	No mutagenic effects.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations	None given
Remarks for results	Orange oil was inactive in *Salmonella* strains TA 98 & TA 100 both in the presence and absence of metabolic activation

	both in the presence and absence of metabolic activation system.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Crebelli R., Aquilina G., Conti L. and Carere A. (1990) Microbial mutagenicity screening of natural flavoring substances. Microbiologica 13, 115-119.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Mouse Lymphoma Forward Mutation Assay (MLY)
Test Type	Forward mutation
System of Testing	Mammalian
GLP	Ambiguous
Year	1990
Species/Strain	L5178Y Mouse Lymphoma cell line
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	100 micrograms/ml
Remarks for Test Conditions	Cells were exposed for four hours, and then washed and incubated at 37 degrees for 48 hours before cloning. Colonies were counted after 10-14 days growth using an automatic colony counter.
Results	No mutagenic effects.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations	None given
Remarks for results	Limonene was negative for mutagenic activity.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Heck J. D., Vollmuth, T. A., Cifone, M. A., Jagannath, D. R., Myhr B., and R.D. Curren (1989). An evaluation of food flavoring ingredients in a genetic toxicity screening battery The Toxicologist, 9(1), 257.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	Mouse Lymphoma Forward Mutation Assay (MLY).
Test Type	Forward mutation
System of Testing	Mammalian
GLP	Ambiguous
Year	1989
Species/Strain	L5178Y Mouse Lymphoma cell line
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	0-100 micrograms/ml
Remarks for Test Conditions	Cells were exposed for four hours, and then washed and incubated at 37 °C for 48 hours before cloning. Colonies were counted after 9-12 days growth using an automatic colony counter.
Results	No mutagenic effects.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations	None given
Remarks for results	Limonene was negative for mutagenic activity.
Conclusion Remarks	No evidence of mutagenicity.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Myhr B., McGregor D., Bowers L., Riach C., Brown A.G., Edwards I., McBride D., Martin R., and Caspary W.J. (1990) L5178Y Mouse Lymphoma Cell Mutation Assay Results With 41 Compounds. Environmental and Molecular Mutagenesis. 16, 138-167.

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)
CAS No.	8008-57-9
Method/guideline	Mouse Lymphoma Forward Mutation Assay (MLY)
Test Type	Forward mutation

System of Testing	Mammalian
GLP	Ambiguous
Year	1990
Species/Strain	L5178Y Mouse Lymphoma cell line
Metabolic Activation	With and without rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	40-120 micrograms/ml, 125-200 micrograms/ml
Remarks for Test Conditions	Cells were exposed for four hours, and then washed and incubated at 37 degrees for 48 hours before cloning. Colonies were counted after 10-14 days growth using an automatic colony counter.
Results	Positive responses were observed with and without S-9, the latter only at highly toxic concentrations (relative survival less than 10%).
Cytotoxic concentration	Not given
Genotoxic Effects	Positive
Appropriate statistical evaluations	None given
Remarks for results	Orange oil was positive with and without S-9.
Conclusion Remarks	The authors stated that the low pH associated with the material may have contributed to the positive outcome. The authors recommended additional assays to clarify the positive response in the MLY assay.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Heck J. D., Vollmuth, T. A., Cifone, M. A., Jagannath, D. R., Myhr B., and R.D. Curren (1989). An evaluation of food flavoring ingredients in a genetic toxicity screening battery The Toxicologist, 9(1), 257.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Sister Chromatid Exchange in Chinese hamster ovary cells (Galloway *et al.,* 1985) with minor modifications
Test Type	Sister Chromatid Exchange
System of Testing	Mammalian
GLP	Ambiguous
Year	1990
Species/Strain	Chinese hamster ovary cells

Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats
Doses/Concentration	16.2-162 micrograms/ml limonene
Statistical Methods	Trend test (Margolin *et al*., 1986)
Remarks for Test Conditions	The standard protocol as published by Galloway *et al*. was employed with minor modifications. Limonene was tested with and without activation. S-9 was used at a concentration of 15 or 20 microliters per milliliters.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	Negative
Appropriate statistical evaluations	Yes
Remarks for results	Negative
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Anderson B.E., Zeiger E., Shelby M.D., Resnick M.A., Gulati D.K., Ivett J.L., and Loveday K.S. (1990) Chromosome aberration and Sister Chromatid Exchange Test Results with 42 chemicals. Environmental and Molecular Mutagenesis 16, 55-137.

Substance Name	Myrcene
CAS No.	123-35-3
Remarks for Substance	*beta*-Myrcene
Method/guideline	In Vitro Sister Chromatid Exchange Test with Human Lymphocytes performed according to (Preston *et al*., 1987)
Test Type	Sister Chromatid Exchange
System of Testing	Mammalian
GLP	Ambiguous
Year	1991
Species/Strain	Human lymphocytes
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats
Doses/Concentration	Up to 1000 micrograms/ml
Statistical Methods	Not given
Remarks for Test Conditions	Peripheral blood samples were obtained from one male and one female non-smoker. The test substances were added after 48 hr either for a period of 24 hours (without S-9 mix) or for a

	period of 2 hours (with S-9 mix). Myrcene was dissolved in ethanol. The mitotic index was determined for 1000 cells and given as number of mitoses per 1000 cells.
Results	No increase in frequency of sister chromatid exchange with or without metabolic activation.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations	None given
Remarks for results	Negative
Conclusion Remarks	The test substance was reported to have reduced the SCE inducing effect of S9 mix activated cyclophosphamide in human lymphocytes in a dose-dependent manner.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Kauderer B., Zamith H., Paumgartten J.R., and Speit G. (1991) Evaluation of the mutagenicity of *beta*-myrcene in mammalian cells in vitro. Environmental and Molecular Mutagenesis 18:28-34.

Substance Name	Myrcene
CAS No.	123-35-3
Remarks for Substance	*beta*-Myrcene
Method/guideline	In Vitro Sister Chromatid Exchange Test with Chinese Hamster Ovary V79 and HTC cells
Test Type	Sister Chromatid Exchange
System of Testing	Mammalian
GLP	Ambiguous
Year	1991
Species/Strain	Chinese hamster ovary cells
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats
Doses/Concentration	Up to 500 micrograms/ml
Statistical Methods	Not given
Remarks for Test Conditions	V79 cells were exposed to myrcene for three hours in the presence or absence of S-9 mix. Hepatic tumour cell (HTC) line cells were cultivated for the duration of one cell cycle in bromodeoxyuridine containing medium and for another 20 hours in bromodeoxyuridine free medium. HTC cells were treated with the test substances during the first cell cycle (20 h).
Results	Negative

Cytotoxic concentration	Not given
Genotoxic Effects	Negative
Appropriate statistical evaluations	Not given
Remarks for results	Negative
Conclusion Remarks	The test substance was reported to have reduced the SCE inducing effect of S9 mix activated cyclophosphamide and alfatoxin B1 in V79 and HTC cells in a dose-dependent manner.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Roscheisen C., Zamith H., Paumgartten F., and Speit G. (1991) Influence of *beta*-myrcene on sister chromatid exchanges induced by mutagens in V79 and HTC cells. Mutation Research 264, 43-49.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Chromosome Aberration in Chinese hamster ovary cells (Galloway *et al*., 1985) with minor modifications
Test Type	Chromosomal Aberration assay
System of Testing	Mammalian
GLP	Ambiguous
Year	1990
Species/Strain	Chinese hamster ovary cells
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats
Doses/Concentration	50-500 micrograms/ml
Statistical Methods	Trend test (Margolin *et al*., 1986)
Remarks for Test Conditions	The standard protocol as published by Galloway *et al*. was employed with minor modifications. Limonene was tested with and without activation. S-9 was used at a concentration of 15 or 20 microliters per milliliters.
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	Negative
Appropriate statistical evaluations	Yes
Remarks for results	Negative

Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Anderson B.E., Zeiger E., Shelby M.D., Resnick M.A., Gulati D.K., Ivett J.L., and Loveday K.S. (1990) Chromosome aberration and Sister Chromatid Exchange Test Results with 42 chemicals. Environmental and Molecular Mutagenesis 16, 55-137.

Substance Name	Myrcene
CAS No.	123-35-3
Remarks for Substance	*beta*-Myrcene
Method/guideline	*In Vitro* Chromosome Aberration Test with Human Lymphocytes performed according to (Preston *et al*., 1987)
Test Type	Chromosomal Aberration assay
System of Testing	Mammalian
GLP	Ambiguous
Year	1991
Species/Strain	Human lymphocytes
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats
Doses/Concentration	Up to 1000 micrograms/ml
Statistical Methods	Not given
Remarks for Test Conditions	Peripheral blood samples were obtained from one male and one female non-smoker. The test substances were added after 48 hr either for a period of 24 hours (without S-9 mix) or for a period of 2 hours (with S-9 mix). Myrcene was dissolved in ethanol. The mitotic index was determined for 1000 cells and given as number of mitoses per 1000 cells.
Results	No induction of chromosome aberrations with or without metabolic activation. No indication of cytotoxicity.
Cytotoxic concentration	Not given
Genotoxic Effects	None
Appropriate statistical evaluations	None given
Remarks for results	Negative
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Kauderer B., Zamith H., Paumgartten J.R., and Speit G. (1991) Evaluation of the mutagenicity of *beta*-myrcene in mammalian cells in vitro. Environ. and Molecular Mutagenesis 18:28-34.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Cell Transformation in Syrian Hamster Embryo Cells
Test Type	Cell Transformation and Effect on Gap Junction Intercellular Communication
System of Testing	Mammalian
GLP	Ambiguous
Year	2000
Species/Strain	Syrian Hamster Embryo Cells
Metabolic Activation	None
Doses/Concentration	0.1-3 millimolar for Cell Transformation and 0.01-1 millimolar for Cellular Communication
Statistical Methods	One sided chi-square and ANOVA
Remarks for Test Conditions	Primary cell cultures from Syrian hamster embryos were prepared at 14 days gestation. The cells were exposed for 7 days starting 1 day after seeding of the target cells. Cells were observed for morphological transformation. Gap junction intercellular communication was measured as spreading of microinjected Lucifer Yellow dye to neighboring cells in a monolayer after 4 hours of exposure to test substances. The numbers of dye-coupled cells were counted 5-8 minutes following injection.
Results	Cell Transformation- 0.13% transformation frequency with a p-value of 0.089 using a chi-square. Cell Communication-No apparent effects
Cytotoxic concentration	Not given
Genotoxic Effects	Not applicable
Appropriate statistical evaluations	Yes
Remarks for results	Not statistically significant for cell transformation in Syrian Hamsters at the *alpha* = 0.05 level. No effect on cellular communication.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Rivedal E., Mikalsen S.O., and Sanner T. (2000) Morphological transformation and effect on gap junction intercellular communication in Syrian Hamster Embryo Cellsas Screening Tests for Carcinogens Devoid of Mutagenic Activity.

Substance Name	*d*-Limonene

CAS No.	5989-27-5
Method/guideline	Cell Transformation in Syrian Hamster Embryo Cells
Test Type	Cell Transformation
System of Testing	Mammalian
GLP	No
Year	1980
Species/Strain	Syrian Hamster Embryo Cells
Metabolic Activation	None
Doses/Concentration	0.1-100 micrograms/ml
Statistical Methods	Not given
Results	Negative
Cytotoxic concentration	Not given
Genotoxic Effects	Negative
Appropriate statistical evaluations	None
Remarks for results	Negative
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Pienta R.J. (1980) Evaluation and relevance of the syrian hamster embryo cell system. The Predictive Value of Short Term Screening Tests in Carcinogenicity Evaluation, 149-160.

Substance Name	Myrcene
CAS No.	123-35-3
Remarks for Substance	*beta*-Myrcene
Method/guideline	V79-HPRT Gene Mutation Test with CHO cells according to Li *et al*., 1987
Test Type	Gene mutation
System of Testing	Mammalian
GLP	Ambiguous
Year	1991
Species/Strain	Chinese hamster ovary cells
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced Sprague Dawley rats

Doses/Concentration	Up to 1000 micrograms/ml
Statistical Methods	Not given
Remarks for Test Conditions	The cells were exposed to myrcene or the control substances for 3 hours in the presence or absence of S-9 mix.
Results	No increase in mutation frequencies at the hprt-locus in V79 cells. No indication of induced cytotoxicity.
Cytotoxic concentration	Non-toxic up to 1000 microrams/ml
Genotoxic Effects	None
Appropriate statistical evaluations	None given
Remarks for results	Negative
Conclusion Remarks	The test substance was reported to have toxic and mutagenic effect of cyclophosphamide in V79 cells.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Kauderer B., Zamith H., Paumgartten J.R., and Speit G. (1991) Evaluation of the mutagenicity of *beta*-myrcene in mammalian cells in vitro. Environmental and Molecular Mutagenesis 18:28-34.

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)
CAS No.	8008-57-9
Method/guideline	*Bacillus subtillis* recessive assay
Test Type	Reverse mutation test
System of Testing	Bacterial
GLP	Ambiguous
Year	1989
Species/Strain	*Bacillus subtilis*
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/Concentration	5-30 microliters/plate
Results	No mutagenic effects.
Cytotoxic concentration	Not given
Genotoxic Effects	Negative
Appropriate statistical evaluations	None given
Remarks for results	Orange oil was negative with or without S-9 activation.

Data Qualities Reliabilities	Reliability code 3. Not reliable.
Remarks for Data Reliability	Code 3. Documentation insufficient for assessment.
References	Kuroda K., Yoo S., Ishibashi T. (1989) Rec-assay of natural food additives. Seikatsu Eisei 33, 15-23.

4.2.2 In vivo Genotoxicity

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Mammalian spot test
GLP	Ambiguous
Year	1984
Species/Strain	Mouse
Sex	Not reported
Route of Administration	Intraperitoneal
Doses/Concentration	215 mg/kg
Exposure Period	One day
Remarks for Test Conditions	Mouse embryos were treated in utero with limonene on days 10 and 11 post conception.
Appropriate statistical evaluations?	Yes, t-test
Genotoxic effects	No effects
Remarks for Results	No effects
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Fahrig R. (1984) Genetic mode of action of carcinogens and tumor promoters in yeast and mice. Molecular and General Genetics 194, 7-14.

Substance Name	Myrcene
CAS No.	123-35-3
Remarks for Substance	*beta*-Myrcene
Method/guideline	*in vivo* Cytogenetic Bone Marrow Assay

GLP	Ambiguous
Year	1993
Species/Strain	Rat/Wistar
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/Concentration	100, 500 or 1000 mg/kg
Exposure Period	24 and 48 hours
Remarks for Test Conditions	*beta*-Myrcene (100, 500 or 1000 mg/kg) was orally administered via gavage to two or four male and female Wistar rats. Corn oil was used as the negative control while cyclophosphamide (30 mg/kg via intraperitoneal injection) was used as the positive control. A mitotic inhibitor (colchicine 5 mg/kg ip) was injected 1 hr before sacrifice. At 24 or 48 hours, animals were sacrificed and bone marrow cells harvested. Evaluations included the mitotic index and the frequency of chromosomal aberrations.
Appropriate statistical evaluations?	Yes, Kruskal-Wallis test, Mann-Whitney test
Effect on mitotic index or PCE/NCE ratio by dose level and sex	Corn oil (24 hr sampling) Number Sex Mitotic index 2 Males 11.5 2 Females 12.5 4 Males and Females 12.0 +/- 2.9 *beta*-Myrcene 100 mg/kg bw (24 hr) 2 Males 14.0 2 Females 17.0 4 Males and Females 15.5 +/- 8.5 500 mg/kg bw (24 hr) 2 Males 19.5 2 Females 19 4 Males and Females 19.2 +/-1.7 1000 mg/kg (24 hr) 4 Males 21.0 4 Females 23.0 4 Males and Females 22.0 +/- 6.1 1000 mg/kg bw (48 hr) 2 Males 16.5 2 Females 12.0 4 Males and Females 14.2 +/-3.6 Cyclophosphamide (24 hr) 30 mg/kg ip 2 Males 10.5 2 Females 8.5 4 Males and Females 9.5+/- 3.7

	2 Females 4 Males and Females
Genotoxic effects	Dose related increase in mitotic index, no statistically significant increases in frequency of chromosomal aberrations
Remarks for Results	A dose-related increase in the mitotic index in bone marrow cells was reported for rats administered *beta*-myrcene. The authors commented that this be an interaction between *beta*-myrcene, which is known to induce CYP-P450 enzymes, and colchicine, which arrests cell division at metaphase. Myrcene may have increased the bioavailability of colchicine leading to the increase in mitotic index observed in the experiment. No significant increases in chromosomal aberrations were reported in the treated animals at either 24 or 48 hours.
Conclusion Remarks	The authors concluded that given the results, *beta*-myrcene was not clastogenic to the rat when orally administered at dose levels up to 1000 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Zamith H.P., Vidal M.N.P., Speit G. and Paumgartten F.J.R. (1993) Absence of genotoxic activity of *beta*-myrcene in the in vivo cytogenetic bone marrow assay. Brazilian J Med Biol Res 26, 93-98.

4.3 Repeat dose Toxicity

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	Greater than 99% pure
Method/guideline	National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 347
GLP	Yes
Year	1990
Species/strain	B6C3F1 Mice
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 413, 825, 1650, 3300, or 6600 mg/kg bw/d
Exposure Period	16 days
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes

Post Exposure	4 days
Remarks for Test Conditions	Groups of five mice of each sex were administered 0, 413, 825, 1650, 3300, or 6600 mg/kg *d*-limonene in corn oil by gavage once per day for 12 days over a 16 day period. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals.
NOAEL (NOEL)	1650 mg/kg bw/d
LOAEL(LOEL)	3300 mg/kg bw/d
Toxic Response/effects by Dose Level	See remarks for results.
Statistical Evaluation	Yes
Remarks for Results	All but one animal receiving 3300 or 6600 mg/kg bw/d limonene died within three days of study initiation. No treatment-related clinical signs were observed in mice receiving doses of 1650 mg/kg bw/d or lower.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP) (1990) Carcinogenicity and toxicology studies of *d*-limonene in F344/N Rats and B6C3F1 mice. NTP-TR-347. U.S. Dept of Health and Human Services. NIH Publication No. 90-2802.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	Greater than 99% pure
Method/guideline	National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 347
GLP	Yes
Year	1990
Species/strain	F344/N Rats
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 413, 825, 1650, 3300, or 6600 mg/kg bw/d
Exposure Period	16 days
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Post Exposure	4 days

Remarks for Test Conditions	Groups of five rats of each sex were administered 0, 413, 825, 1650, 3300, or 6600 mg/kg *d*-limonene in corn oil by gavage once per day for 12 days over a 16 day period. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals.
NOAEL (NOEL)	1650 mg/kg bw/d
LOAEL(LOEL)	3300 mg/kg bw/d
Toxic Response/effects by Dose Level	All but two females receiving 3300 or 6600 mg/kg bw/d limonene died within two days of study initiation. No treatment related clinical signs were observed in rats receiving doses of 1650 mg/kg bw/d or lower.
Statistical Evaluation	Yes
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP) (1990) Carcinogenicity and toxicology studies of *d*-limonene in F344/N Rats and B6C3F1 mice. NTP-TR-347. U.S. Dept of Health and Human Services. NIH Publication No. 90-2802.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	Greater than 99% pure
Method/guideline	National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 347
GLP	Yes
Year	1990
Species/strain	F344/N Rats
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 150, 300, 600, 1200, or 2400 mg/kg bw/d
Exposure Period	13 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Remarks for Test Conditions	Groups of ten rats of each sex were administered 0, 150, 300, 600, 1200 or 2400 mg/kg bw/d *d*-limonene in corn oil by gavage once per day, five days a week for 13 weeks. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals. Histological

	examinations were performed on all vehicle control and high dose animals and all female rats in the 1200 mg/kg group. Tissues examined included adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), femur, sternebrae or vertebrae including marrow, gross lesions and tissue masses with regional lymph nodes, heart kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, small intestine, spinal cord (if neurologic signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Kidneys were examined in all male rats.
NOAEL (NOEL)	300 mg/kg bw/d
LOAEL(LOEL)	600 mg/kg bw/d
Toxic Response/effects by Dose Level	See remarks for results.
Statistical Evaluation	Yes
Remarks for Results	Ninety percent of female rats (9/10) and fifty percent of male rats (5/10) receiving 2400 mg/kg bw/d limonene died within the first week of the study. The final mean body weights of male rats receiving the three highest doses (600, 1200 or 2400 mg/kg bw/d) were reported to be 6%, 12%, or 23% lower than that of the controls, respectively. Rough hair coats, lethargy, and excessive lacrimation were observed for all animals at the two highest dose levels. Nephropathy was reported for all groups of male rats but a dose related increase in severity of the lesion was reported for the dosed groups. The nephropathy was characterized by degeneration of epithelium in the convoluted tubules, granular casts with tubular lumens, primarily in the outer stripe of the outer medulla, and regeneration of the tubular epithelium. Hyaline droplets were observed in the epithelium of the proximal convoluted tubules in all groups of male rats including vehicle controls. Upon further review to determine if there were differences in these findings between control and treated animals, the blinded slides revealed no definite differences in the accumulation of hyaline droplets.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP) (1990) Carcinogenicity and toxicology studies of *d*-limonene in F344/N Rats and B6C3F1 mice. NTP-TR-347. U.S. Dept of Health and Human Services. NIH Publication No. 90-2802.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	Greater than 99% pure

Method/guideline	National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 347
GLP	Yes
Year	1990
Species/strain	B6C3F1 Mice
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 125, 250, 500, 1000 or 2000 mg/kg bw/d
Exposure Period	13 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Remarks for Test Conditions	Groups of ten mice of each sex were administered 0, 125, 250, 500, 1000 or 2000 mg/kg bw/d *d*-limonene in corn oil by gavage once per day, five days a week for 13 weeks. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week. Necropsies were performed on all animals. Histological examinations were performed on all vehicle control and high dose animals. Tissues examined included adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), femur or sternebrae or vertebrae including marrow, gallbladder, gross lesions and tissue masses with regional lymph nodes, heart kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, small intestine, spinal cord (if neurological signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder.
NOAEL (NOEL)	500 mg/kg bw/d
LOAEL(LOEL)	1000 mg/kg bw/d
Toxic Response/effects by Dose Level	One of 10 males and 2/10 females administered 2000 mg/kg bw/d limonene and 1/10 females administered 500 mg/kg bw/d limonene died before the end of the study. Several other animals also died as a result of gavage error. Mean body weights were 10% lower than control for male mice and 2% lower than control for female mice for the two highest dose levels. An alveolar cell adenoma was reported in the lung of one female at the highest dose level. Clinical signs of rough hair coats and decreased activity were reported for the two highest dose levels.
Statistical Evaluation	Yes
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.

References	National Toxicology Program (NTP) (1990) Carcinogenicity and toxicology studies of *d*-limonene in F344/N Rats and B6C3F1 mice. NTP-TR-347. U.S. Dept of Health and Human Services. NIH Publication No. 90-2802.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	*d*-limonene; greater than 99% pure
Method/guideline	Subchronic nephrotoxicity study
GLP	Ambiguous
Year	1989
Species/strain	F344/N Rats
Sex	Male
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 2, 5, 10, 30, or 75 mg/kg bw/d
Exposure Period	13 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Remarks for Test Conditions	Groups of five week old male rats received 0, 2, 5, 10, 30 or 75 mg/kg bw/d *d*-limonene daily via oral gavage for 13 weeks (5 days a week). Rats from selected dose groups were necropsied throughout the study (days 8-29), with all remaining rats necropsied at the end of the study. Rats were observed daily for toxicity signs. Body weights were taken daily.
NOAEL (NOEL)	5 mg/kg bw/d
LOAEL(LOEL)	30 mg/kg bw/d
Toxic Response/effects by Dose Level	5 mg/kg bw/d no effects 10 mg/kg bw/d formation of hyaline droplets 30 mg/kg bw/d increased relative kidney weight, formation of hyaline droplets; 75 mg/kg bw/d increased relative kidney and liver weights; increased granular casts in outer medulla; formation of hyaline droplets.
Statistical Evaluation	Yes, linear regression analyses
Remarks for Results	Linear regression analyses indicated increased relative kidney and liver weights at the two highest dose levels. Histological examination revealed changes characterized by hyaline droplet formation, granular casts and multiple cortical changes, all of which was classified as chronic nephrosis. Exacerbation of hyaline droplet formation was reported at the earliest necropsy eight days after administration at the 10 mg/kg bw/d dose level.

Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Webb D.R., Ridder M., and Alden C.L. (1989) Acute and subchronic nephrotoxicity of *d*-limonene in Fischer 344 rats.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
GLP	No
Year	1977
Species/strain	Rat/Sprague Dawley
Sex	Not reported
Route of Administration	Oral
Doses/concentration Levels	0, 277, 554, 1385, or 2770 mg/kg bw/d
Exposure Period	30 days
Frequency of Treatment	Daily
Control Group	Yes
Remarks for Test Conditions	The test substance was orally administered to Sprague-Dawley rats daily for 30 days at the following dose levels 0, 277, 554, 1385, or 2770 mg/kg bw in order to investigate the effect on the fine structure of the liver, kidney and blood cells.
NOAEL (NOEL)	1385 mg/kg bw/d
LOAEL(LOEL)	2770 mg/kg bw/d
Toxic Response/effects by Dose Level	No morphological changes of renal corpuscles and tubular cells were observed. Some alterations were detected in the glomerular epithelium from the kidneys of rats treated at the highest dose level.
Statistical Evaluation	Not given
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Kodama R., Tahara S., Sato K., Noda K., Ide H., Nishihara H. (1977b) Studies on *d*-limonene as a Gallstone Solubilizer Fine Structure of Liver, Kidneys and Blood Cells from Rats given *d*-limonene. Oyo Yakuri 13(6), 875.

Substance Name	*d*-Limonene
CAS No.	5989-27-5

Remarks for Substance	Greater than 99% pure
Method/guideline	National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 347
GLP	Yes
Year	1990
Species/strain	F344/N Rats
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	Males: 0, 75 or 150 mg/kg bw/d; Females: 0, 300 or 600 mg/kg bw/d
Exposure Period	103 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Remarks for Test Conditions	Groups of fifty male and fifty female rats each were administered 0, 75 or 150 mg/kg bw/d or 0, 300 or 600 mg/kg bw/d *d*-limonene, respectively, in corn oil by gavage once per day, five days a week for 103 weeks. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study; all vehicle control; all low dose female rats and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland. Tissues examined in low dose male rat groups included adrenal glands, kidney, liver, spleen, and testis.
NOAEL (NOEL)	Undetermined (male); 300 mg/kg bw/d (female)
LOAEL(LOEL)	75 mg/kg bw/d (male); 600 mg/kg bw/d (female)
Toxic Response/effects by Dose Level	Male Rats Body weight-5% reduction in high dose group. Survival rate-29/50; 33/50;40/50. Non-neoplastic effects-mineralization 7/50; 43/50; 48/50; epithelial hyperplasia 0/50; 35/50; 43/50 of the renal papilla; renal tubular cell hyperplasia 0/50; 4/50; 7/50. Neoplastic effects-renal tubular cell adenomas 0/50; 4/50; 8/50 an adenocarcinomas 0/50; 4/50; 3/50.

	Female Rats Body weight-5% reduction in high dose group. Survival rate-42/50; 40/50;26/50. No neoplastic or nonneoplastic effects.
Statistical Evaluation	Yes
Remarks for results	Mean body weights for male rats administered 150 mg/kg bw/d *d*-limonene were generally 4-7% lower than vehicle controls from week 2 to study termination. Mean body weights of high dose females were generally 4-7% lower than vehicle controls from week 28 to study termination. No treatment related clinical signs were reported for the duration of the study. Survival of the high dose male group was significantly greater than that of the vehicle alone after week 81. Survival of the high dose female group was significantly lower than that of the vehicle controls after week 39. In the kidneys of male rats, dose-related increases in the incidences of mineralization and epithelial hyperplasia. A dose-related increase in the severity of spontaneous nephropathy was reported in male rats administered limonene. Increased incidence of tubular cell hyperplasia and neoplasia was also reported in dosed male rats. Tubular cell adenoma incidence in high dose male rats and tubular cell adenoma or tubular cell carcinomas (combined) in dosed male rats were significantly greater than vehicle controls.
Conclusion Remarks	The authors determined that under the conditions of these 2-year gavage studies there was clear evidence of carcinogenic activity of *d*-limonene for male F344/N rats as shown by increased incidences in tubular cell hyperplasia, adenomas, and adeno-carcinomas of the kidney. There was no evidence of carcinogenic activity of *d*-limonene for female rats receiving 300 or 600 mg/kg bw/d. It has been demonstrated that renal lesions, which were observed in the NTP study, resulted from the accumulation of aggregates of a-2m-globulin (a low molecular-weight protein synthesized in the liver) and limonene or its metabolites in the P2 segment of the renal proximal tubule. This phenomenon has only been observed in the male F344/N rat [Strasser, 1988; Borghoff *et al*., 1990]. The gene that encodes a-2m-globulin has been isolated and the sequence deduced [Untermann *et al*., 1981]. These proteins are expressed in the liver under hormonal control [Roy and Neuhaus, 1967; Wang and Hodgetts, 1998]. *a*-2m-Globulin belongs to the a-2m-globulin super family of proteins that are characterized by a unique hydrophobic binding pocket. The lesions do not develop in the female F344/N rat or in humans [Bucher *et al*., 1986]. Subsequent investigations have shown that the a-2m-globulin nephropathy found in the F344/N male rat does not develop in mammals that do not express the hepatic form of a-2m-globulin [Swenberg, 1989] such as other strains of rats [Dietrich and Swenberg, 1991], mice [Bucher *et al*., 1986; Lehman-McKeeman, 1994] and dogs [Webb, 1990].

Transgenic mice that express rat a-2m-globulin were tested for their ability to form hyaline droplets and develop nephropathies similar to their adult male rat counterparts [Lehman-McKeeman and Caudill, 1994]. This study involved male F344 rats as positive control, transgenic C57BL/6J mice as experimental group and native C57BL/6 mice as negative controls. The animals at age 70-75 days were placed in metabolic cages and received 150 mg/kg bw per day *d*-limonene in corn oil by gavage for three days. Limonene was used to induce renal nephropathy in adult male rats, as it was shown to be a potent inducer in the NTP studies [EPA, 1991; NTP, 1990]. Twenty-four (24) hours after the last dose, the animals were sacrificed and the kidneys analyzed for evidence of nephropathy. Hyaline droplet formation was evaluated on a subjective scale, size and intensity (0-4) multiplied by tubular loading (0-3) for an overall scale of 0-12 with 12 being the most severe. In the absence of *d*-limonene, the control groups transgenic mice and rats showed a hyaline droplet score of 1+/- 0 and 6 +/- 0.5, respectively. The test transgenic mice and rats showed a hyaline droplet score of 2.5 +/- 0.3 and 11 +/- 1.3, respectively upon dosing with *d*-limonene. The native mice developed no signs of hyaline droplet formation and tested negative for presence of a-2m-globulin in their urine. The authors assert that based on the data presented "a-2m-globulin is the only protein that is involved in the etiology of hyaline droplet nephropathy".

An increase in the kidney-type-a-2m-globulin was seen in the urine of male Sprague-Dawley rats when these animals were administered greater than 30 mg/kg/day of *d*-limonene for 7 days by gavage. The increases in the urinary kidney-type-a-2m-globulin are dose-dependent and parallel-elevated accumulation in the kidney cells [Saito, 1996]

In another study, adult male Wistar rats were administered two groups of chemical compounds, including 138 mg/kg bw isophorone, potassium bromate, 2-propanol and a series of benzene and anthracene derivatives, to study induction of accumulation of a-2m-globulin and structure-activity relationships. A monoclonal antibody against a-2m-globulin was employed in a competitive ELISA procedure to determine its concentration in urine or tissue samples without purification. Plasma concentrations of a-2m-globulin were not significantly increased by any of the test compounds at 1 mmol/kg bw. Kidney tissue concentrations were found to be 297-300% higher than controls. The hyaline droplet accumulating (HDA) potential was dependent on the test compound but there was no relationship between HAD activity and the structure or the pathway used to metabolize the test substance [Hildebrand, 1997]

While humans produce low molecular weight serum proteins, which are reabsorbed by the kidney, there is no evidence that a-2m-globulin is produced [Olson, 1990]. Urine collected from adult male F344 rats and humans revealed no evidence indicative of a-2m-globulin production in humans [Olson, 1990].

	It is unknown whether any human serum proteins possess a binding site similar to that of a-2m-globulin. Although this is a possibility, it appears remote, since female rats and mice do not show the renal changes noted in male rats exposed to limonene. It should be noted that there is a class of human proteins referred to as the a-2m-globulin-related proteins. They appear to have no functional relationship to the adult male rat urine proteins. The human protein has a higher molecular weight, 25 kDa and is a component of a neutrophil gelatinase complex [Kjeldsen *et al*., 2000 and Triebel *et al*., 1992]. An extensive review of the current scientific literature and genome databases reveals no native protein or biological entity that acts as a nephropathy agent like mature male rat a-2m-globulin. The accumulated evidence indicates that it is the unique anatomical, physiological, and biochemical properties of the male rat kidney, especially the proximal convoluted tubule, that allows *d*-limonene to interfere with renal processing of the strain-specific a-2m-globulin. Therefore, this process is not predictive of human carcinogenicity. In a comprehensive review of a-2m-globulin nephropathy and associated renal tubule tumors produced in the male F344/N rat exposed to limonene and other simple chemical substances (e.g. isophorone, decalin and methyl isobutyl ketone), it was concluded that the F344/N rat is not an appropriate model for assessing human renal carcinogenic risk [EPA, 1991]. After careful review, it has been concluded that the mechanisms leading to the renal carcinogenic findings in the F344/N male rat are largely known and strongly indicate that the nephropathy associated with *d*-limonene have no significance for human risk assessment [Burdock *et al*., 1990].
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP) (1990) Carcinogenicity and toxicology studies of *d*-limonene in F344/N Rats and B6C3F1 mice. NTP-TR-347. U.S. Dept of Health and Human Services. NIH Publication No. 90-2802.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	Greater than 99% pure
Method/guideline	National Toxicology Program. Toxicology and Carcinogenesis study NTP TR 347
GLP	Yes
Year	1990
Species/strain	B6C3F1 Mice
Sex	Male and Female

Route of Administration	Oral-Gavage
Doses/concentration Levels	Males: 0, 250 or 500 mg/kg bw/d; Females: 0, 500 or 1000 mg/kg bw/d
Exposure Period	103 weeks
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes
Remarks for Test Conditions	Groups of fifty male and fifty female mice each were administered 0, 250, or 500 mg/kg bw/d or 0, 500 or 1000 mg/kg bw/d *d*-limonene, respectively, in corn oil by gavage once per day, five days a week for 103 weeks. Animals were housed five per cage and fed ad libitum. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Necropsies were performed on all animals. Histological examinations were performed on all animals dying during the study, all vehicle controls, and all high dose animals. Tissues examined included adrenal glands, brain, cecum, colon, costochondral junction, duodenum, epididymus/seminal vesicles/tunica vaginalis/scrotal sac/prostrate/testes or ovaries/uterus, esophagus, eyes, femur or sternebrae or vertebrae including marrow, gallbladder, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs and bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroids, pituitary gland, preputial or clitoral gland, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, urinary bladder and Zymbal gland. Tissues examined in low dose groups include liver for female mice.
NOAEL (NOEL)	500 mg/kg bw/d
LOAEL(LOEL)	Undetermined for males; 1000 mg/kg bw/d for female
Toxic Response/effects by Dose Level	Male mice Body weight- No effect. Survival rate-24/50;44/50;39/50. Nonneoplastic effects-No effects. Neoplastic effects-No effects. Female mice Body weight- 10% reduction in high dose group by study end. Survival rate-43/50;44/50;43/50. Nonneoplastic effects-No effects. Neoplastic effects-No effects.
Statistical Evaluation	Yes
Remarks for Results	Mean body weights for female mice administered 1000 mg/kg bw/d *d*-limonene were generally 5-15% lower than vehicle controls from week 28 to study termination. No treatment related clinical signs were reported for the duration of the study. Survival of the low dose male group was significantly lower than that of the vehicle controls by study termination.

Conclusion Remarks	The authors determined that under the conditions of these 2-year gavage studies there was no evidence of carcinogenic activity of *d*-limonene for male or female B6C3F1 mice at the dose levels tested.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	National Toxicology Program (NTP) (1990) Carcinogenicity and toxicology studies of *d*-limonene in F344/N Rats and B6C3F1 mice. NTP-TR-347. U.S. Dept of Health and Human Services. NIH Publication No. 90-2802.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
GLP	Ambiguous
Year	1987
Species/strain	F344/N Rats
Sex	Male
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 75, 150, or 300 mg/kg bw/d
Exposure Period	27 days
Frequency of Treatment	Daily (5 days/week)
Control Group	Yes, vehicle only
Remarks for Test Conditions	Groups of five young adult male F344/N rats each were administered *d*-limonene at dose levels of 0, 75, 150 or 300 mg/kg bw/d five days a week for 27 days. Observations included daily body weight, weekly food intake, liver and kidney weights and light microscopy and histology of liver and kidneys. Rats were examined for hyaline drop formation, granular cast formation and chronic nephrosis. Two-dimensional gel electrophoresis evaluation of protein profiles was conducted on samples of kidneys in the 150 mg/kg dose group killed on day 6.
NOAEL (NOEL)	Less than 75 mg/kg bw/d
LOAEL(LOEL)	75 mg/kg bw/d
Toxic Response/effects by Dose Level	Dose related increases in liver and kidney weights were reported for all dose levels. Renal effects were noted including protein profile changes, hyaline droplet formation, and accumulation of *alpha*-2-globulin was reported.
Statistical Evaluation	Yes
Conclusion Remarks	Chronic nephrosis was present in all kidneys of treated animals killed on day 27. The authors noted that unlike female rats or

	higher mammalian species, male rats have anatomical, physiological and biochemical peculiarities involving the proximal convoluted tubule.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Kanerva R.L., Ridder G.M. Lefever F.R. and Alden C.L. (1987) Comparison of short-term renal effects due to oral administration of decalin or *d*-limonene in young adult male Fischer rats. Fd Chem Toxicol 25, 345-353.

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)
CAS No.	8008-57-9
Method/guideline	28-Day Oral Toxicity Study
GLP	Yes
Year	1990
Species/strain	Rat/Sprague Dawley
Sex	Male and Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 240, 600 or 1500 mg/kg bw/d
Exposure Period	30 days
Frequency of Treatment	Daily
Control Group	Yes, vehicle only
Remarks for Test Conditions	Groups of ten rats of each sex were administered 0, 240, 600 or 1500 mg/kg bw/d sweet orange oil in 1% methyl cellulose by gavage daily for 30 days. Observations included survival, clinical observations, body weights, food consumption, clinical pathology, gross pathology, organ weights and histopathology.
NOAEL (NOEL)	Less than 240 mg/kg bw/d
LOAEL(LOEL)	240 mg/kg bw/d
Toxic Response/effects by Dose Level	1500 mg/kg bw/d, Males-
Statistical Evaluation	Yes
Remarks for Results	No treatment related effects were reported for survival, clinical observations, body weights or food consumption. Decreases in glucose related to treatment were reported in the mi*d*-dose females and high dose males and females. Increases in serum albumin and total serum protein were observed in all treated females and the high dose males. Histopathology revealed treatment related lesions in the nonglandular stomach of the high dose males and females and in the kidney of all treated

	male groups. Kidney weights were also increased in the treated male groups and in the high dose female group. Liver weight increases related to treatment were reported for the high-dose females and all dosed male groups.
Conclusion Remarks	The authors concluded that the NOEL under conditions of this study was less than 240 mg/kg bw/d for both male and female rats. The authors noted that the kidney changes observed in the male rat at all dose levels were expected given the known interaction between limonene and *alpha*-2-microglobulin. Limonene is the principal constituent (greater than 90%) of orange oil.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Serota D. (1990) 28-Day oral toxicity study in rats. Unpublished report to FEMA.

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)
CAS No.	8008-57-9
Method/guideline	Immunotoxicity-PFC assay- (Plaque Forming Cell) Assay to sheep red blood cells; Host Resistance Assay- Listeria monocytogenes
GLP	Yes
Year	1994
Species/strain	B6C3F1 mice
Sex	Female
Route of Administration	Oral-Gavage
Doses/concentration Levels	0, 625, 1250 or 2500 mg/kg bw
Exposure Period	5 days
Frequency of Treatment	Daily
Control Group	Yes
Remarks for Test Conditions	Orange oil was administered intragastrically to female B6C3F1 mice daily for 5 days at dose levels of 0, 625, 1250 or 2500 mg/kg bw to determine effects on humoral and cell-mediated immune responses. A host resistance assay (Listeria monocytogenes bacterial challenge) was used to assess cell-mediated immunity while the antibody plaque forming cell response to sheep erythrocytes was used to measure humoral immunity. Other parameters evaluated included body weights, lymphoid organ weights and spleen cellularity but in the absence of modulation of the PFC response, these effects were not considered indicators of immunotoxicity.
NOAEL (NOEL)	2500 mg/kg bw/d

LOAEL(LOEL)	Greater than 2500 mg/kg bw/d
Toxic Response/effects by Dose Level	None
Statistical Evaluation	Yes
Conclusion Remarks	Orange oil had no effects on cell-mediated or humoral immune response at any dose level tested.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Gaworski, C. L., Vollmuth, T. A., Dosier, M. M., Heck, J. D., Dunn, L. T., Ratajczak, H. V. and Thomas, P. T. (1994). An Immunotoxicity Assessment of Food Flavouring Ingredients. Food Chem. Toxicol. 32(5): 409-415.

4.4 Reproductive Toxicity

Substance Name	Myrcene
CAS No.	123-35-3
Remarks for Substance	*beta*-Myrcene
Method/guideline	Not given
GLP	Ambiguous
Year	1993
Species/Strain	Rat/Wistar
Sex	Female
Route of Administration	Oral
Duration of Test	Approximately 128 days
Doses/Concentration	250, 500, 1000 or 1500 mg/kg bw/d
Premating Exposure period for males	Not applicable
Premating Exposure period for females	27, 28 or 29 days depending upon delivery date
Control Group and Treatment	Yes, vehicle only (corn oil at 2.5 ml/kg bw)
Frequency of Treatment	Daily
Remarks for Test Conditions	*beta*-Myrcene was administered to female Wistar rats via gavage at dose levels of 0, 250, 500, 1000 or 1500 mg/kg bw/d from the 15th day of gestation until weaning of the offspring which was day 21 postnatal. The vehicle was corn oil. Mortality, weight gain and postnatal development were

	evaluated. Reproductive capacity was assessed in the exposed offspring upon reaching maturity (120 days).
NOAEL(NOEL)	250 mg/kg bw/d
LOAEL(LOEL)	500 mg/kg bw/d
Appropriate statistical evaluations	One way ANOVA and student t test
Remarks for Results	No adverse effects were noted in the offspring at the lowest dose level tested. Decreased body weight, increased perinatal mortality, and delayed developmental landmarks were noted at the 500, 1000 and 1500 mg/kg bw/d dose levels. Fertility was impaired in female offspring exposed to the two highest doses of *beta*-myrcene.
Data Reliabilities Qualities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Delgado I.F., Nogueira A. C., Souza C.M., Costa M.N., Figueiredo L.H., Mattos A.P., Chahoud I. And Paumgartten F. (1993b) Peri- and postnatal developmental toxicity of *beta*-myrcene in the rat. Fd Chem Toxic 31, 623-628.

Substance Name	Myrcene
CAS No.	123-35-3
Remarks for Substance	*beta*-Myrcene
Method/guideline	Not given
GLP	Ambiguous
Year	1998
Species/Strain	Rat/Wistar
Sex	Male and Female
Route of Administration	Oral-Gavage
Duration of Test	Approximately 86 days for female animals; approximately 112 days for male animals
Doses/Concentration	100,300, or 500 mg/kg bw/d
Premating Exposure period for males	91 days
Premating Exposure period for females	21 days
Control Group and Treatment	Yes, vehicle only (peanut oil at 2.5 ml/kg bw)
Frequency of Treatment	Daily
Remarks for Test Conditions	Three experimental groups (15 male and 45 female Wistar rats per group) were administered *beta*-myrcene dissolved in peanut oil via gavage at dose levels of 0, 100, 300, or 500 mg/kg bw/d. The exposure period was 91 days prior to and

	during mating the mating period for the males and 21days prior to and during the mating period for females, pregnancy, and lactation until 21 days post parturition. All parent animals were evaluated for weight development, mortality, and toxicity signs. Pregnant females were also evaluated for weight gain, spontaneous abortions, dystocia and prolonged duration of pregnancy. All males were sacrificed and decapitated at the conclusion of mating. One third of the females in each dose group were sacrificed at day 21 of pregnancy. The gravid uterus weight was recorded; resorption and living and dead fetuses were counted. Implantation sites were counted. All fetuses were examined for skeletal abnormalities. After the remaining pregnant females gave birth, the offspring was weighed, and examined for signs of developmental delays, specifically, incisor eruption, fur development, downy hair development, and eye opening. At weaning on day 21, all mothers were sacrificed and necropsied.
NOAEL(NOEL)	300 mg/kg bw/d
LOAEL(LOEL)	500 mg/kg bw/d
Appropriate statistical evaluations	Yes, one way ANOVA, two tailed student t test
Parental data and F1 as Appropriate	No deaths or signs of toxicity were reported in male rats at any dose level. No statistically significant differences in body weight gain were reported between control and test animals. A slight increase in liver and kidney weights was reported for treated male (highest dose only) and female rats. No morphological alterations of the liver or testis tissue were revealed upon examination. No effects were reported on the number of spermatids in the testis or on the number of spermatozoa in the cauda epididymis. No adverse effects on body weight gain and no other signs of toxicity were observed in treated female rats during the premating or mating periods. No treatment related effects were reported on fertility as measured by the mating index and pregnancy index upon comparison to controls. At the highest dose level, a slight increase in the resorption rate and a parallel decrease in the ratio of live fetuses per implantation site were reported.
Offspring toxicity F1 and F2	Increases in the occurrence of fetal skeleton abnormalities between control and treated groups were reported at the 500 mg/kg bw/d level. No adverse effects were reported on duration of pregnancy, labor, pup mortality, and maternal or offspring weight changes. Slight delays in incisor eruption (300 mg/kg bw/d) and eye opening (100, 300 mg/kg bw/d) were reported but were not dose-related.
Remarks for Results	The authors attributed the increase in skeletal abnormalities at the highest dose level tested to known strain-specific anomalies including increases in dislocated sternums, and lumbar extra ribs.
Conclusion Remarks	The authors concluded that the NOAEL for toxic effects on fertility and general reproductive performance via the oral route was 300 mg *beta*-myrcene/kg bw/d.
Data Reliabilities Qualities	Reliability code 2. Reliable with restriction.

Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Paumgartten F.J., De-Carvalho R.R., Souza C.C., Madi K. and Chahoud I. (1998) Study of the effects of *beta*-Myrcene on rat fertility and general reproductive performance. Braz J Med Biol Res, 31(7).

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)
CAS No.	8008-57-9
Method/guideline	*in vivo* Reproductive and Developmental Toxicity Screening Test
GLP	Yes
Year	1989
Species/Strain	Rat/Sprague Dawley
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	39 days
Doses/Concentration	0, 375, 750 or 1500 mg/kg bw/d
Premating Exposure period for males	Not applicable
Premating Exposure period for females	7 days
Control Group and Treatment	Yes, vehicle only (corn oil)
Frequency of Treatment	Daily
Remarks for Test Conditions	Groups of ten female rats were orally administered sweet orange oil via gavage at dose levels of 0, 375, 750 or 1500 mg/kg bw/d for seven days prior to and through cohabitation, gestation, delivery and a four day lactation period. The vehicle was corn oil. Body weights, food consumption and clinical signs were recorded throughout the observation period. All dams were necropsied and examined for gross lesions on day 25 of presumed gestation for rats not delivering a litter and four days postpartum for rats delivering a litter. Pups delivered were sacrificed on day 4 post partum, any pups dying during the lactation period were necropsied.
NOAEL(NOEL)	Less than 375 mg/kg bw/d
LOAEL(LOEL)	375 mg/kg bw/d
Appropriate statistical evaluations	Yes
Parental data and F1 as Appropriate	No deaths occurred at any dose level. Statistically significant numbers of rats from all three dose groups experienced excess salvation during the pre-mating and gestation periods, and during the lactation period for high-dose animals. The dosed

	rats had decreased weight gains compared to the control rats during the seven day premating period. Absolute and relative maternal food consumption was significantly decreased for the 750 and 1500 mg/kg bw/d dose groups during the seven day premating period. No treatment related effect on mating performance or fertility was reported at any dose level.
Offspring toxicity F1 and F2	A significant increase in stillbirths and pup deaths was reported for the highest dose group compared to the control group. The treatment with sweet orange oil had no effect on the incidence of malformations or gross lesions in the pups.
Conclusion Remarks	The NOAEL for administration of sweet orange peel oil under the conditions of this study was reported to be less than 375 mg/kg bw/d for maternal toxicity and 750 mg/kg bw/d for offspring development.
Data Reliabilities Qualities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Hoberman A.M., Vollmuth T.A., Bennett M.B. and M.S. Christian (1989) An evaluation of food flavoring ingredients using an in vivo reproductive and developmental toxicity screening test. Private communication.

4.5 Developmental/Teratogenicity Toxicity

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Not given
GLP	Ambiguous
Year	1975
Species/strain	Rat/Wistar
Sex	Female
Route of Administration	Oral
Duration of Test	7 days
Doses/concentration Levels	0, 591 or 2869 mg/kg bw/d
Exposure Period	Daily from day 9 to 15 of gestation
Frequency of Treatment	Daily
Control Group and Treatment	Yes
Remarks for Test Conditions	Four groups of twenty Wistar female rats each were administered 0, 591 or 2869 mg/kg bw/d *d*-limonene on days 9-15 of gestation.

NOAEL(NOEL) maternal toxicity	591 mg/kg bw/d
LOAEL(LOEL) maternal toxicity	2869 mg/kg bw/d
NOAEL (NOEL) developmental toxicity	591 mg/kg bw/d
LOAEL (LOEL) developmental toxicity	2869 mg/kg bw/d
Actual dose received by dose level and sex	Not given
Maternal data with dose level	2869 mg/kg bw/d- maternal body weight decreased, and increased mortality was reported
Fetal Data with Dose Level	2869 mg/kg bw/d-delayed ossification of fetuses metacarpal bone and proximal phalanx; decreased body weights; decreased weights of thymus, spleen, and ovaries.
Appropriate statistical evaluations	Not given
Remarks for Results	At the highest dose level, increases in maternal mortality and decreases in maternal and fetal body weights were reported. Additionally at the highest dose level, delayed ossification of fetal metacarpal bones and proximals phlanx and decreased weights of the thymus, spleen, and ovaries were reported.
Conclusion Remarks	The NOAEL for both maternal and offspring toxicity was reported to be 591 mg/kg bw/d.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Tsuji M., Y.Fujisaki, Y.Arikawa, S.Masuda, S.Kinoshita, A.Okubo, K.Noda, H.Ide and Y.Iwanaga (1975b) Studies on *d*-limonene as a gallstone solubilizer: Effects on Development of Rat Fetuses and Offsprings. Journal Oyo Yakuri, 10(2), 179.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Remarks for Substance	*d*-Limonene
Method/guideline	Not given
GLP	Ambiguous
Year	1977
Species/strain	Rabbits/Japanese White
Sex	Female
Route of Administration	Oral
Duration of Test	13 days
Doses/concentration Levels	0, 250, 500 or 1000 mg/kg bw/d

Exposure Period	Daily from day 6 to 18 of gestation
Frequency of Treatment	Daily
Control Group and Treatment	Yes
Remarks for Test Conditions	Pregnant Japanese white rabbits were administered 0, 250, 500 or 1000 mg/kg bw/d *d*-limonene on days 6 to 18 of gestation.
NOAEL(NOEL) maternal toxicity	250 mg/kg bw/d
LOAEL(LOEL) maternal toxicity	500 mg/kg bw/d
NOAEL (NOEL) developmental toxicity	Greater than 1000 mg/kg bw/d
LOAEL (LOEL) developmental toxicity	Not determined
Actual dose received by dose level and sex	Not given
Maternal data with dose level	1000 mg/kg bw/*d*-increased mortality; 500 and 1000 mg/kg bw/*d*-significant decreases in body weight gain and food consumption temporarily observed
Fetal Data with Dose Level	No treatment related effects reported.
Appropriate statistical evaluations	Not given
Remarks for Results	Increased maternal mortality was reported at the highest dose level. Significant decreases in maternal body weight gain and food consumption were temporarily observed at the 500 and 1000 mg/kg bw/d dose levels. No treatment related effects were reported for the offspring.
Conclusion Remarks	The NOAEL for maternal toxicity was 250 mg/kg bw/d. The NOAEL for offspring toxicity was greater than 1000 mg/kg bw/d.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Kodama R., Okubo A., Sato K., Araki E., Noda K., Ide H., and Ikeda T. (1977a) Studies on *d*-limonene as a gallstone solubilizer: Effect on development of rabbit fetuses and offsprings.Journal Oyo Yakuri, 13(6), 885-898.

Substance Name	*d*-Limonene
CAS No.	5989-27-5
Method/guideline	Not given
GLP	Ambiguous
Year	1977
Species/strain	Mice/ICR
Sex	Female

Route of Administration	Oral
Duration of Test	6 days
Doses/concentration Levels	0, 591 or 2363 mg/kg bw/d
Exposure Period	Daily from day 7 to 12 of gestation
Frequency of Treatment	Daily
Control Group and Treatment	Yes
Remarks for Test Conditions	Pregnant ICR mice were administered 0, 591 or 2363 mg/kg bw/d *d*-limonene on days 7 to 12 of gestation.
NOAEL(NOEL) maternal toxicity	591 mg/kg bw/d
LOAEL(LOEL) maternal toxicity	2363 mg/kg bw/d
NOAEL (NOEL) developmental toxicity	591 mg/kg bw/d
LOAEL (LOEL) developmental toxicity	2363 mg/kg bw/d
Actual dose received by dose level and sex	Not given
Maternal data with dose level	2363 mg/kg bw/*d*-significant decrease of body weight gain
Fetal Data with Dose Level	2363 mg/kg bw/*d*-increased incidence of fused ribs compared to control; delayed ossification of some bones, which returned to normal after birth; significant decrease in body weight gain of male offspring
Appropriate statistical evaluations	Not given
Remarks for Results	Significant decreases in body weight gain were reported for pregnant ICR mice administered the highest dose level of *d*-limonene. In the offspring, increased incidence of fused ribs compared to that of the controls, delayed ossification of some bones and decreased body weight gain were reported at the highest dose level tested.
Conclusion Remarks	The NOAEL for both maternal and offspring toxicity was reported to be 591 mg/kg bw/d.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Kodama R., Okubo A., Sato K., Araki E., Noda K., Ide H., and Ikeda T. (1977a) Studies on *d*-limonene as a gallstone solubilizer: Effect on development of mouse fetuses and offsprings.Journal Oyo Yakuri, 13(6), 885-898.

Substance Name	Myrcene
CAS No.	123-35-3
Remarks for Substance	*beta*-Myrcene

Method/guideline	Not given
GLP	Ambiguous
Year	1993
Species/strain	Rat/Wistar
Sex	Female
Route of Administration	Oral
Duration of Test	10 days
Doses/concentration Levels	0, 250, 500 or 1200 mg/kg bw/d
Exposure Period	Daily from day 6 to 15 of gestation
Frequency of Treatment	Daily
Control Group and Treatment	Yes
Remarks for Test Conditions	Pregnant Wistar rats were administered 0, 250, 500 or 1200 mg/kg bw/d *beta*-myrcene on gestational days 6-15. The vehicle was corn oil.
NOAEL(NOEL) maternal toxicity	500 mg/kg bw/d
LOAEL(LOEL) maternal toxicity	1200 mg/kg bw/d
NOAEL (NOEL) developmental toxicity	500 mg/kg bw/d
LOAEL (LOEL) developmental toxicity	1200 mg/kg bw/d
Actual dose received by dose level and sex	Not given
Maternal data with dose level	1200 mg/kg bw/d-1/29 maternal deaths. Decreased maternal weight gain.
Fetal Data with Dose Level	1200 mg/kg bw/d-Increased skeletal malformations.
Appropriate statistical evaluations	Yes
Remarks for Results	Decreased maternal weight gain was reported at the 1200 mg/kg bw/d dose. Increased fetal skeletal malformations were reported at the 1200 mg/kg bw/d dose level.
Conclusion Remarks	The NOAEL for both maternal and offspring toxicity was reported to be 500 mg/kg bw/d.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Delgado I.F., Carvalho R.R., Nogueira A., Mattos A.P., Figueiredo L.H., Oliveira S.H.P., Chahoud I., and Paumgartten F.J.R. (1993a) Study on embryo-fetotoxicity of *beta*-myrcene in the rat. Food Chem Toxic 31, 31-35.

Substance Name	Myrcene
CAS No.	123-35-3
Remarks for Substance	*beta*-Myrcene
Method/guideline	Not given
GLP	Ambiguous
Year	1993
Species/strain	Rat/Wistar
Sex	Female
Route of Administration	Oral
Duration of Test	Approximately 128 days
Doses/concentration Levels	0, 250, 500, 1000 or 1500 mg/kg bw/d
Exposure Period	27, 28 or 29 days depending upon delivery date
Frequency of Treatment	Daily
Control Group and Treatment	Yes, vehicle only (corn oil at 2.5 ml/kg bw)
Remarks for Test Conditions	*beta*-Myrcene was administered via gavage to female Wistar rats from pregnancy day 15 until weaning of the offspring which was day 21 postnatal. The vehicle was corn oil. Mortality, weight gain and postnatal development were evaluated. Reproductive capacity was assessed in the exposed offspring upon reaching maturity (120 days).
NOAEL(NOEL) maternal toxicity	500 mg/kg bw/d
LOAEL(LOEL) maternal toxicity	1000 mg/kg bw/d
NOAEL (NOEL) developmental toxicity	250 mg/kg bw/d
LOAEL (LOEL) developmental toxicity	500 mg/kg bw/d
Actual dose received by dose level and sex	Not given
Maternal data with dose level	1500 mg/kg bw/d: 5/15 deaths within first four days of treatment; decreased weight. 1000 mg/kg bw/d: No maternal deaths. Weight deficit observed at term no longer present at parturition.
Fetal Data with Dose Level	500, 1000, or 1500 mg/kg bw/d: decreased body weight, increased perinatal mortality and delayed developmental landmarks; impaired fertility (1000 and 1500 mg/kg bw/d only)
Appropriate statistical evaluations	One way ANOVA and student t test
Remarks for Results	No adverse effects were noted in the offspring at the lowest dose level tested. Decreased body weight, increased perinatal mortality, and delayed developmental landmarks were noted at the 500, 1000 and 1500 mg/kg bw/d dose levels. Fertility was

	impaired in female offspring exposed to the two highest doses of *beta*-myrcene.
Conclusion Remarks	The NOAEL for peri- and post natal development was set at 250 mg/kg bw/d.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Delgado I.F., Nogueira A. C., Souza C.M., Costa M.N., Figueiredo L.H., Mattos A.P., Chahoud I. And Paumgartten F. (1993b) Peri- and postnatal developmental toxicity of *beta*-myrcene in the rat. Fd Chem Toxic 31, 623-628.

Substance Name	Orange peel oil, sweet (Citrus sinensis (L.) Osbeck)
CAS No.	8008-57-9
Method/guideline	*in vivo* Reproductive and Developmental Toxicity Screening Test
GLP	Yes
Year	1989
Species/strain	Rat/Sprague Dawley
Sex	Female
Route of Administration	Oral-Gavage
Duration of Test	39 days
Doses/concentration Levels	0, 375, 750 or 1500 mg/kg bw/d
Exposure Period	38 days
Frequency of Treatment	Daily
Control Group and Treatment	Yes, vehicle only (corn oil)
Remarks for Test Conditions	Groups of ten female rats were orally administered sweet orange oil via gavage at dose levels of 0, 375, 750 or 1500, 375 mg/kg bw/d for seven days prior to and through cohabitation, gestation, delivery and a four day lactation period. The vehicle was corn oil. Body weights, food consumption and clinical signs were recorded throughout the observation period. All dams were necropsied and examined for gross lesions on Day 25 of presumed gestation for rats not delivering a litter and four days postpartum for rats delivering a litter. Pups delivered were sacrificed on day 4 post partum, any pups dying during the lactation period were necropsied.
NOAEL(NOEL) maternal toxicity	Less than 375 mg/kg bw/d
LOAEL(LOEL) maternal toxicity	375 mg/kg bw/d
NOAEL (NOEL) developmental toxicity	750 mg/kg bw/d

LOAEL (LOEL) developmental toxicity	1500 mg/kg bw/d
Maternal data with dose level	No deaths occurred at any dose level. Statistically significant numbers of rats from all three dose groups experienced excess salvation during the premating and gestation periods, and during the lactation period for high dose animals. The dosed rats had decreased weight gains compared to the control rats during the seven-day premating period. Absolute and relative maternal food consumption was significantly decreased for the 750 and 1500 mg/kg bw/d dose groups during the seven day premating period. No treatment related effects were reported on maternal body weight, changes in body weight, and absolute and relative feed consumption during the lactation period.
Fetal Data with Dose Level	A significant increase in stillbirths and pup deaths was reported for the highest dose group compared to the control group. The treatment with sweet orange oil had no effect on the incidence of malformations or gross lesions in the pups.
Appropriate statistical evaluations	Yes
Conclusion Remarks	The NOAEL for administration of sweet orange peel oil under the conditions of this study was reported to be less than 375 mg/kg bw/d for maternal toxicity and 750 mg/kg bw/d for offspring development.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Hoberman A.M., Vollmuth T.A., Bennett M.B. and M.S. Christian (1989) An evaluation of food flavoring ingredients using an in vivo reproductive and developmental toxicity screening test. Private communication.