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The Flavor And Fragrance High Production Volume Consortia

The Terpene Consortium

Revised Test Plan For Bicyclic Terpene Hydrocarbons

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***alpha*-Pinene**	**CAS No. 80-56-8**
***beta*-Pinene**	**CAS No. 127-91-3**
Camphene	**CAS No. 79-92-5**
***cis*-Pinane**	**CAS No. 6876-13-7**
Dihydropinene	**CAS No. 473-55-2**
***l-alpha*-Pinene**	**CAS No. 7785-26-4**
Terpenes & Terpenoids, Turpentine oil, *alpha*-Pinene	**CAS No. 65996-96-5**
Terpenes & Terpenoids, Turpentine oil, *beta*-Pinene	**CAS No. 65996-97-6**
Turpentine gum	**CAS No. 9005-90-7**
Turpentine oil	**CAS No. 8006-64-2**

FFHPVC Terpene Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:
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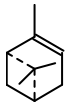
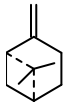


alpha-Pinene
$C_{10}H_{16}$

Synonyms:
Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-
Pin-2(3)-en
2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene

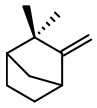
CAS No. 80-56-8



beta-Pinene
$C_{10}H_{16}$

Synonyms:
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-
Pin-2(10)-ene
6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane

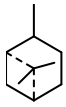
CAS No. 127-91-3



Camphene
$C_{10}H_{16}$

Synonyms:
Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-
2,2-Dimethyl-3-methylenebicyclo[2.2.1]heptane

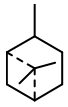
CAS No. 79-92-5



cis-Pinane
$C_{10}H_{18}$

Synonyms:
Bicyclo[3.1.1]heptane,2,6,6-trimethyl-, (1a,2b,5a)-
(1a,2b,5a)-2,6,6-Trimethylbicyclo[3.1.1]heptane

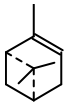
CAS No. 6876-13-7



Dihydropinene
$C_{10}H_{18}$

Synonyms:
Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-
2,6,6-Trimethylbicyclo[3.1.1]heptane

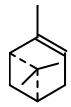
CAS No. 473-55-2



l-alpha-Pinene
$C_{10}H_{16}$

Synonyms:
Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (1S)-
(-)-Pin-2(3)-ene
(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene

CAS No. 7785-26-4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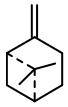
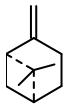


92-97% 1-7%

Terpenes and terpenoids, turpentine oil, *alpha*-pinene fraction
Synonyms: Oil of turpentine, *alpha*-pinene fraction
CAS No. 65996-96-5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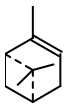
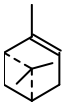

+ other terpene hydrocarbons

78-81% 8-10%

Terpenes and terpenoids, turpentine oil, *beta*-pinene fraction
Synonyms: Oil of turpentine, *beta*-pinene fraction
CAS No. 65996-97-6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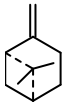
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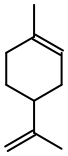
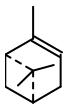

+ other terpene hydrocarbons

59% 24% 5%

Turpentine oil
Synonyms: Oil of turpentine,Turpentine
CAS No. 8006-64-2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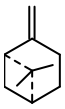

+ 5-8% other monocyclic terpene hydrocarbons

60-65% 25-35%

Turpentine gum
Synonyms: Turpentine
CAS No. 9005-90-7

2 Category Analysis

2.1 Introduction

In October of 1999, members of the United States 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 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 the Flavor and Fragrance High Production Volume Consortia 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 test plan, category analysis, 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 "Bicyclic Terpene Hydrocarbons" includes six simple bicyclic terpene hydrocarbons and four mixtures comprised primarily of the six bicyclic terpene hydrocarbons. These terpene hydrocarbons are extracted from plants, mainly from the resins of suitable *Pinus* species of trees. The role bicyclic terpene hydrocarbons play in the biology of plants and animals is significant. *alpha*-Pinene, a principal member of this category, is one of the most common C_{10} terpene hydrocarbons in nature. Combinations of terpenes such as *alpha*-pinene and *alpha*-terpineol have bactericidal

activity while oxygenated terpene hydrocarbons formed from *alpha*-pinene (*e.g.* verbenol and myrtenal) act as natural insect pheromones.

Natural Occurrence in Food

In plants, bicyclic terpene hydrocarbons, such as *alpha*-pinene, are produced by the isoprene pathway, an integral part of normal plant biosynthesis. a*lpha*-Pinene, *beta*-pinene, pinane, camphene, and *delta*-3-carene are therefore, ubiquitous in the plant kingdom. They are common components of traditional foods occurring in essentially all fruits and vegetables [CIVO-TNO, 1999]. a*lpha*-Pinene, *beta*-pinene, pinane, *delta*-3-carene, and camphene 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 1,000,000 pounds (lbs) of pinene (*alpha* and *beta*-pinene combined) are consumed annually as components of food in the United States. In fact, greater than a 1,000,000 lbs. of alpha-pinene is consume annually as a constituent of black pepper [Stofberg and Grundschober, 1987]. Less than 50,000 lbs are consumed as added flavoring substances [Stofberg and Grundschober, 1987]. Therefore, greater than 95% of oral intake occurs from consumption of food containing naturally occurring pinene. Based on the annual volume of consumption of pinene, it is estimated that the combined average daily *per capita* intake of *alpha*- and *beta*-pinene is approximately 10 mg/day. Intakes as high as 100 mg/day ("eaters only"- 90 percentile intake) [Oser and Hall, 1977] may be expected for consumers of diets rich in spices, fruits, and vegetables. Given that the essential oils of spices such as nutmeg, pepper, and marjoram are rich in alpha- and beta-pinene and other $C_{10}H_{16}$ bicyclic hydrocarbons such as sabinene and camphene [Bauer and Garbe, 1985], it would be expected that heavy "spice eaters" are in the "eaters only" group.

Natural Occurrence as Atmospheric Emissions

As a volatile C_{10} hydrocarbon, pinene is also a naturally occurring component of the atmosphere. Estimates of atmospheric concentrations of *alpha*- and *beta*-pinene 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 5-10 ug/m^3 [Koistinen *et al*., 1998; Samfield, 1992; Krause *et al.*, 1987], 500-1200 ug/m^3 [Kodama *et al.*, 1977], and 200,000-500,000 ug/ m^3, respectively [Sittig, 1977]. The Occupational Safety and Health Administration (OSHA) permissible exposure level (PEL) [Occupational Health and Environmental Control, 1974] and threshold limit value (TLV) [American Conference of Governmental Industrial Hygienists, 1993] for turpentine composed mainly of *alpha*- and *beta*-pinene is 560,000 ug/m^3. This permissible level of exposure is at least 1000 times the urban or rural atmospheric concentrations of *alpha*- and *beta*-pinene. Assuming that a human is exposed daily to an urban atmosphere containing 10 ug/m^3 *alpha*-pinene and that 60% of the inhaled *alpha*-pinene is absorbed [Falk *et al.*, 1990], the daily intake from atmospheric exposure would be approximately 0.7 mg/day 1{10 ug/m^3 x 3 m^3/hr x 24hrs/day x 0.6 (absorption rate) x 10^{-3} ug/mg}. In a rural environment in which atmospheric concentrations of *alpha*-pinene are routinely 500 ug/m^3, daily inhalation intake may approach 20 mg/day. When oral and inhalation exposures are combined, it is estimated that average total daily exposure from food consumption and normal inhalation in an urban or rural environment is in the range from 10-30 mg. However, for specialized eating groups (90 percentile of eaters, *e.g.,* spice and vegetable eaters), average daily intake may easily exceed 100 mg.

Industrial Extraction of Bicyclic Terpene Hydrocarbons from Plant Sources

The major industrial source of bicyclic terpene hydrocarbons is crude sulfate turpentine (CST) obtained from wood pulp as a waste product in the manufacturer of cellulose via the sulfate process. A minor source of bicyclic terpene hydrocarbons is wood turpentine that is obtained by the steam distillation of chopped tree trunks and dead wood. The principal constituents of all turpentine oils (CST and wood turpentine) are bicyclic terpene hydrocarbons. CST obtained from southern paper mills in the United States

consists mainly of *alpha*-pinene (60-70%) and *beta*-pinene (20-25%) together with small amounts of limonene (3-10%), anethole (1-2%), and aliphatic tertiary alcohols (3-7%). In western mills, CST is composed of smaller amounts of *beta*-pinene (3-11%), but proportionately higher levels of a structurally related bicyclic hydrocarbon *delta*-3-carene (1-38%) together with a mixture of aliphatic tertiary alcohols (8-20%) [Derfer and Traynor, 1992]. Russian and Scandinavian turpentine oil is rich in *delta*-3-carene and camphene.

2.3 Structural Classification

The chemical category designated bicyclic terpene hydrocarbons includes six simple bicyclic terpene hydrocarbons and four mixtures composed primarily of *alpha*- and *beta*-pinene and smaller amounts of the other four chemically identified terpene hydrocarbons. *alpha*-Pinene and *beta*-pinene are bicyclic monounsaturated terpenes and are positional isomers of each other. *alpha*-Pinene is 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene while *beta*-pinene is 2-methylene-6,6-dimethylbicyclo [3.1.1]heptane. *l*-*alpha*-Pinene is the *l*-stereoisomer of *alpha*-pinene. *cis*-Pinane and dihydropinene are the saturated derivatives of *alpha*- and *beta*-pinene. They differ only in that *cis*-pinane is one of two diasterisomers of dihydropinene in which the 2-methyl is *cis*- with respect to the geminal dimethyl moiety. Camphene is also a bicyclic terpene hydrocarbon with a [2.2.1] carbon skeleton. Camphene is 3-methylene-2,2-dimethylbicyclo[2.2.1] heptane. Camphene is structurally related to *beta*-pinene in that both are bicyclic C_{10} hydrocarbons that contain an exocyclic methylene function. A structurally related C_{10} terpene bicyclic hydrocarbon found in many essential oils is sabinene or 2-methylene-5-isopropylbicyclo[3.1.0]hexane.

Of the six bicyclic terpene hydrocarbons in this chemical category, *alpha*-pinene is, by far, the most widely occurring in nature. Mixtures of (+)- and (-)- *alpha*-pinene occur in varying ratios in 60-80% concentration in American gum, sulfate, and wood turpentines. The (+)-isomer can be found in concentrations up to 95% in Turkish turpentine while the (-)-isomer predominates (90-96%) in Spanish and Austrian turpentines [Bauer and Garbe, 1985].

The four mixtures in this chemical category are all composed primarily of *alpha*- and *beta*-pinene. The combined concentration of *alpha*- and *beta*-pinene in each of these four mixtures exceeds 80%. The remaining fraction is accounted for mainly by other terpene bicyclic (camphene and carene), monocyclic (*e.g.,* limonene) and monoaromatic hydrocarbons (*e.g., p*-cymene) and terpene tertiary alcohols. Two of these mixtures are distillation products of turpentine oil. One fraction (CAS No.65996-96-5) is rich in *alpha*-pinene (92–97%) and the other (CAS No. 65996-97-6) rich in *beta*-pinene (78–81%). The other two mixtures are both variations on the common naturally occurring solvent, turpentine, varying only slightly in composition as a result of their methods of preparations. A typical analysis of turpentine oil (CAS No. 8006-64-2) includes 59% *alpha*-pinene, 24% *beta*-pinene, 5% dipentene (racemic limonene), 2% each *beta*-phellandrene, *alpha*-terpineol, and linalool, 1% each methyl chavicol, *cis*-anethole, *trans*-anethole [Arizona Chemical, 1999]. Turpentine gum (CAS No. 9005-90-7) is composed of 60-65% *alpha*-pinene, 25-35% *beta*-pinene, and 5-8% monocyclic terpenes (limonene, etc.) [Derfer and Traynor, 1992].

2.4 Industrial and Biogenic Production

2.4.1 Industrial Production

Turpentine is a clear liquid composed of approximately 60-65% *alpha*-pinene, 25-35% *beta*-pinene with the remainder being other monocyclic terpenes such as limonene. 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 crude sulphate turpentine [National Resources Institute, 1995]. In 1977, the annual United States production of CST and wood turpentine was reported to be 92,750 and 9,150 tons, respectively [McKibben, 1979]. Turpentine is derived primarily from *Pinus* species. Turpentine is used in whole form as a solvent for paints and varnishes or as a cleaning agent. Its major use however is as a source material for *alpha*-pinene- or *beta*-pinene-enriched fractions as well as the purified compounds themselves.

The purified *alpha*- and *beta*-pinenes are themselves flavor and fragrance ingredients [Hall and Oser, 1965; Opdyke, 1978]. However, by far the largest uses of *alpha*- and *beta*-pinene and the two enriched fractions are as starting materials in the synthesis of a wide range of other flavor and fragrance ingredients, such as linalool and geraniol, and medicinal products such as vitamins A and E.

Since the 1960's, natural sources of terpenes could no longer meet the world-wide demand for terpenes used in flavors, fragrances, cosmetics, vitamins, and medicines, and household products. *alpha*-Pinene and *beta*-pinene, being the primary constituents of CST produced by the paper industry, have now become the raw materials for the production of many of the commercially important terpenes. Today, *alpha*-pinene and *beta*-pinene are used to produce the vast majority of the terpenes derivatives with annual production volumes greater than 1,000,000 lbs (i.e., terpineol, linalool, linalyl acetate, nerol, geraniol, and citral).

Level-one fugacity calculations indicate that the environmental distribution of turpentine and its components is essentially entirely into the air [Trent University, 1999]. If it were conservatively assumed that through the various industrial processes approximately 2% is lost, the total annual worldwide emission of turpentine and its bicyclic hydrocarbon components would be 6,600 metric tons. This can be compared with the biogenic emissions into the air discussed below.

While discharge into the primary effluent of a pulp and paper mill has been demonstrated for *alpha*-pinene, *beta*-pinene and camphene [Koistinen *et al*., 1998] there were no detectable levels of these materials in the secondary effluent or sludge indicating a high degree of biodegradation.

2.4.2 Biogenic Production

In a recent review article [Guenther *et al*., 2000] it was said, "Natural emissions of volatile compounds are an important component of the earth system responsible for determining the composition of the atmosphere." All of the substances in this group are

relatively volatile and are widely naturally occurring in plants, especially conifers [Helmig *et al.,* 1999a]. Measurements of emissions from sixty-three vegetation species in this study reported the occurrence of *alpha*-pinene, *beta*-pinene and camphene so commonly as to lead to the conclusion that these materials are practically ubiquitous in plants. In determining the impact on the environment of the industrial production and use of the materials in this group, it is also important to examine the impact as a result of emissions from biogenic sources [Guenther *et al*., 2000].

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, *delta*-3-carene, *beta*-pinene and camphene with *alpha*-pinene greatly dominating, 57-73%. Interestingly, it has been shown that both R and S stereoisomers of *alpha*-pinene, *beta*-pinene and camphene can be found in various parts of *Pinus sylvestris* and *Picea abies* trees [Sjödin *et al*., 2000].

By no means are the emissions *alpha*-pinene, *beta*-pinene and camphene limited to conifers. In a study of emissions over arable crops and a beech forest [Gallagher *et al*., 2000] all three were detected and some species of Mediterranean Oak have been shown to be major emitters of *alpha*- and *beta*-pinene with lesser amounts of camphene [Csiky and Seufert, 1999]. 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 United States from each of 63 species of trees [Helmig *et al*., 1999a, 1999b]. All three substances were detected in a substantial proportion of the species measured with fluxes ranging from 0.1 to 80 $\mu gChr^{-1}gdw^{-1}$ (micrograms carbon per hour per gram dry weight) for *alpha*-pinene, 0.1 to 24 $\mu gChr^{-1}gdw^{-1}$ for *beta*-pinene and 0.2 to 39 $\mu gChr^{-1}$ gdw^{-1} for camphene [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 *alpha*-pinene these were 57, 150 and 39 $\mu gCm^{-2}hr^{-1}$ (micrograms carbon per m^2 per hour), for *beta*-pinene, 9, 55 and 3 $\mu gCm^{-2}hr^{-1}$ and for camphene, 32, 130 and 12 $\mu gCm^{-2}hr^{-1}$. These emissions amounted to, for *alpha*-pinene, 2.8, 3.3 and 1.6%, for *beta*-pinene, 0.4, 1.2 and 0.1% and for camphene, 1.6, 2.9 and 0.5% 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 *alpha*-pinene, *beta*-pinene and camphene was 4.5, 3.2 and about 0.1 million metric tons, respectively. 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 1150 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, 2.8, 3.3 and 1.6% for *alpha*-pinene (average = 2.6%), 0.4, 1.2 and 0.1% (0.57%) for *beta*-pinene and 1.6, 2.9 and 0.5% (1.7%) for camphene, it can be estimated that the total annual global emissions for these three substances would be approximately 30 million, 6.5 million and 19 million metric tons respectively. On the other hand, if the average rates of emission of *alpha*-pinene (82 $\mu gCm^{-2}hr^{-1}$), *beta*-pinene (22 $\mu gCm^{-2}hr^{-1}$) and camphene (58 $\mu gCm^{-2}hr^{-1}$) 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 approximately 28 million, 7.5 million and 20 million metric tons for *alpha*-pinene, *beta*-pinene and camphene, respectively, can be calculated.

Based on the above estimates, it can be concluded that total annual atmospheric emission of *alpha*-pinene, *beta*-pinene, and camphene 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 *alpha*-pinene, *beta*-pinene, camphene, or other C_{10} hydrocarbons, the GER would exceed 1000, suggesting that biogenic emissions far exceed man-made emissions. As a result, humans are unavoidably exposed to naturally occurring bicyclic terpene hydrocarbons.

2.5 Chemical Reactivity and Metabolism

As the principal C_{10} hydrocarbon component released into the atmosphere by plants, *alpha*-pinene is rapidly absorbed by animals, distributed, metabolized to polar

oxygenated metabolites and eliminated in the urine and exhaled air. As components of a traditional diet, *alpha*-pinene and *beta*-pinene, *l*-*alpha*--pinene, *cis*-pinane, dihydropinane, and camphene are also rapidly absorbed from the gut, metabolized and excreted primarily in the urine and, to a minor extent, in the feces. Rodent and humans studies for *alpha*-pinene, *beta*-pinene, (-)-*cis*-pinane and structurally related bicyclic terpene hydrocarbons such as *delta*-3-carene indicate that the hydrocarbons in this chemical category participate in similar pathways of absorption, metabolism to polar oxygenated metabolites, and excretion.

2.5.1 Inhalation Pharmacokinetics in Humans

The tissue distribution, metabolism, and excretion of *alpha*-pinene have been studied in human volunteers *via* inhalation. Human volunteers were exposed to an atmosphere containing 0,10, 225, or 450 mg/m^3 of (+)-*alpha*-pinene [Falk *et al.*, 1990] or *delta*-3-carene [Falk *et al.*, 1991] for 2 hours in an exposure chamber on four occasions. Volunteers exercised on a bicycle ergometer during exposure. Total pulmonary uptake of (+)-*alpha*-pinene increased linearly with dose with 40% and 58% uptake occurring at 10 mg/m^3 and 450 mg/m^3, respectively. Uptake of *delta*-3-carene was 61% and 70% at 10 mg/m^3 and 450 mg/m^3, respectively. There was no difference in pulmonary uptake between the enantiomers (+)- and (-)-*alpha*-pinene at 450 mg/m^3. Clearance of *alpha*-pinene and *delta*-3-carene from the blood was rapid (1.1 and 0.9 L/hr/kg, respectively) indicating that *alpha*-pinene and *delta*-3-carene are rapidly metabolized. Blood levels of either substance at the two lower doses were below detection limits 4 hours after exposure. Elimination was considered triphasic with (+) and (-)-*alpha*-pinene exhibiting a rapid initial appearance phase (4.8 and 5.6 minutes, respectively), a rapid elimination phase (30 and 48 minutes, respectively) and a slow elimination phase (695 and 555 minutes, respectively). A long half-life in poorly perfused tissues indicates high affinity for adipose tissue. It was estimated that it would require 2 and 6 days to completely eliminate *alpha*-pinene and *delta*-3-carene, respectively, from the body. Less than 0.001% of the total uptake of *alpha*-pinene or *delta*-3-carene was eliminated unchanged in the urine during and immediately after exposure. There was no evidence of changes in

acute lung function during or 20 minutes after exposure to *alpha*-pinene and *delta*-3-carene. The authors concluded that short-term exposure to relatively high atmospheric concentrations (greater than 100,000 mg/m^3) of *alpha*-pinene did not result in acute changes to lung function under exercising conditions.

Five humans were exposed for 4 or 6 hours to an atmospheres containing 6.4 or 3.2 ppm (24 mg/m^3 and 12 mg/m^3) of a mixture of volatile organic substances. At 6.4 ppm, the air concentration of *alpha*-pinene was 0.139 ppm (0.775 mg/m^3). The mean pre-exposure blood concentration of *alpha*-pinene of 0.035 ppb increased to an average concentration 2.0 ppb during exposure (50-240 minutes). Thereafter (330-450 minutes), the mean blood concentration then decreased to 0.15 ppb. At 3.2 ppm exposure, changes proportional to those observed at 6.4 ppm were recorded. Similar results were also recorded for a 6-hour exposure [Ashley and Prah, 1997]. In a similar study, workers exposed for 8 hours to atmospheres containing 0.035, 070, or 0.105 ppm of *alpha*-pinene showed effective blood concentrations (average difference between blood plateau levels and pre-exposure baseline levels) of 0.94, 1.9, or 3.5 ppb [Kawai *et al.*, 1992].

2.5.2 Metabolism in Human

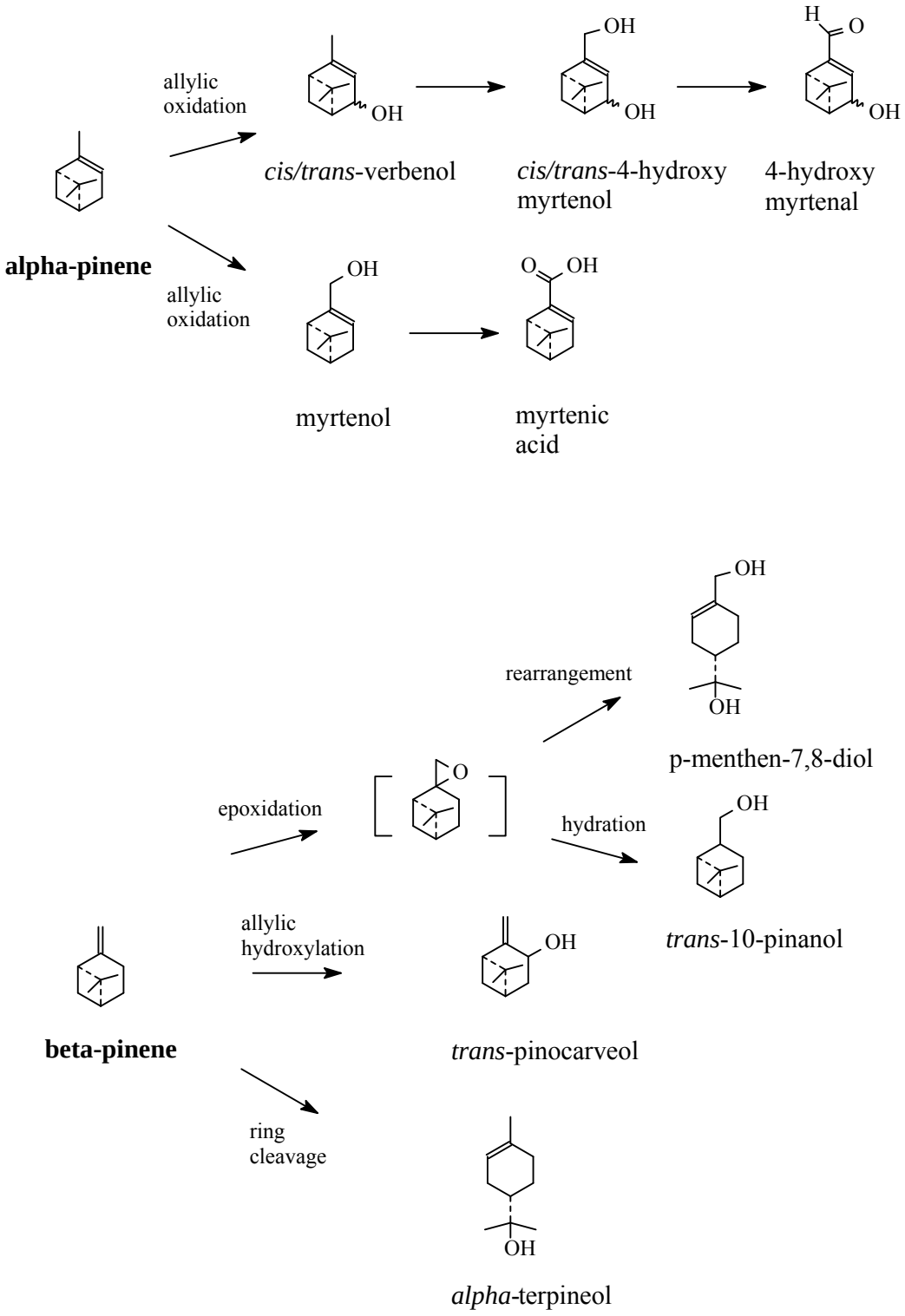
In humans, bicyclic terpene hydrocarbons are metabolized by cytochrome P-450 (CYP-450) induced C-oxidation to produce polar oxygenated metabolites that are conjugated and excreted primarily in the urine (see Figure 1). Analysis of urinary metabolites eliminated within 4 hours following a 2-hour exposure in the above pharmacokinetic study [Falk *et al.*, 1990] revealed *cis*- and *trans*-verbenol in a ratio of 1:10 with 3.8% being eliminated at 10 mg/m^3 and 1.7% at 450 mg/m^3. Most of the verbenols were eliminated within 20 hours. Respiratory elimination of (-)- and (+)-*alpha*-pinene was approximately 8% during exposure [Levin *et al.,* 1992]. Sawmill workers exposed to an atmosphere containing 40-300 mg/m^{-3} for three days showed urinary levels of 10-50 micrograms/ml of *cis* and *trans*-verbenol [Eriksson and Levin, 1990].

In a more extensive metabolic study, urine was collected from sawmill workers at the end of an 8-9 hour work shift or from chamber-exposed individuals. Following hydrolysis of

glucuronic acid conjugates, *cis*- and *trans*-verbenol were identified in the urine along with two diols, *cis*- and *trans*-4-hydroxymyrtenol, formed by methyl group hydroxylation of *cis*- and *trans*-verbenol. *trans*-4-Hydroxymyrtenal was also detected (see Figure 1) [Eriksson and Levin, 1996].

Analysis of the urinary metabolites of a patient attempting suicide with 400-500 ml pine oil containing 57% *alpha*-pinene showed the presence of myrtenol, verbenol, and borneol. Renal excretion reached a peak level 5 days after ingestion [Koppel *et al.*, 1981]. The urine of normal humans has been shown to contain *alpha*-pinene, *beta*-pinene, *delta*-3-carene, and camphene [Zlatkis *et al.*, 1973].

Figure 1 - Metabolism of alpha- and beta-Pinene in Animals



2.5.3 Metabolism in Other Animals

The metabolic detoxication of bicyclic terpene hydrocarbons in mammals is similar to that in humans. Male albino rabbits (6/group) were given a single oral dose levels of 400-700 mg/kg bw of (+)-*alpha*-pinene, (-)-*alpha*-pinene, (±)-*alpha*-pinene, (-)-*beta*-pinene, (-)-*cis*-pinane, or (+)-*delta*-3-carene. The test substance was administered by stomach tube as a suspension in a water/polysorbate 80. Animals were house individually and urine was collected daily for 3 days. Greater than 80% of each bicyclic terpene hydrocarbon was recovered in the urine as glucuronic acid conjugates of hydroxylated terpene hydrocarbons [Ishida *et al.*, 1981].

The principal metabolite formed by allylic oxidation of the exocyclic methyl group of each of the three {(+), (-), or (±)} stereochemical forms of *alpha*-pinene was verbenol (see Figure 1). Greater than 98% of (-)-*alpha*-pinene was converted to (-)-*trans*-verbenol while 67% of (+)-*alpha*-pinene was converted to racemic *trans*-verbenol. In addition, the (+)-isomer metabolized by allylic oxidation of the C_2 methyl group to yield myrtenol (15%) and small amounts of oxidized myrtenol, myrtenic acid.

The presence of an exocyclic alkene function in (-)-*beta*-pinene provides for additional metabolic options. Allylic oxidation of the C_2 position yields (+)-*trans*-pinocarveol (11%) while epoxidation of the exocyclic alkene followed by reduction or hydration yields (-)-*trans*-10-pinanol (39%) and (-)-*l*-*p*-menthene-1,8-diol (30%), respectively. Ring cleavage yields (-)-*alpha*-terpineol (5%) (see Figure 1).

The absence of an alkene function in (-)-*cis*-pinane and dihydropinene prohibits epoxidation. In this case, (-)-*cis*-pinane undergoes ring hydroxylation yielding 3- and 4-pinanol and verbenol and ring cleavage yielding the tertiary terpenoid alcohols (-)-*alpha*-terpineol (43%) and (-)-*trans*-soberol (6%). In a manner similar to *alpha*-pinene, *delta*-3-carene undergoes oxidation of a ring allylic position followed by ring opening to yield (-)-*m*-mentha-4,6-dien-8-ol (72%). Smaller amounts of metabolites obtained by hydroxylation at the gem-dimethyl group and allylic oxidation of the methyl group are obtained [Ishida *et al.*, 1981].

In the brushtail possum (*T. vulpecula)*, *alpha*-pinene is metabolized to *trans*-verbenol, myrtenol, and myrtenic acid while *beta*-pinene is metabolized primarily to myrtenic acid [Southwell *et al.*, 1980]. Likewise, the *Ips* [Renwick *et al*., 1976] and *Dendroctomus* [Hughes, 1975] bark beetles metabolize *alpha*-pinene to verbenol and myrtenol with verbenol being further oxidized to verbenone in *Dendroctomus*.

The biotransformations of *alpha*-pinene and other members of this chemical category are catalyzed by NADPH-dependent cytochrome P-450. Fractions designated CYP-450 and CYP-451 obtained from rat liver microsomes were incubated with *alpha*-pinene for 1-8 minutes intervals. Analysis of the homogenate revealed the presence of *beta*-pinene and limonene together with smaller amounts of *trans*-verbenol, myrtenol, verbenone, and pinane oxide. The proportion of oxidized metabolites was greater in P-451 than in P-450 [White and Agosin, 1980]. Terpene hydrocarbons have been found to block the metabolic activation of promutagenic substances. Both (-)-*alpha*-pinene and (+)-*alpha*-pinene produced a concentration-dependent inhibition of pentoxyresorufin-O-depentylase (a marker for the isoenzyme CYP-2B1) activity [De-Oliveira *et al.*, 1997].

In summary, humans, throughout their lifetime, are continually exposed to *alpha*-pinene, *beta*-pinene, and other terpene C_{10} hydrocarbons *via* inhalation of air or by consumption of a traditional diet. These aliphatic terpene hydrocarbons are rapidly absorbed *via* the oral route and subsequently undergo first-pass metabolism in the liver. Upon single inhalation exposure, the absorbed hydrocarbons may be distributed to adipose tissue in which case, complete clearance from the body requires days. However, taking into account the continuous intake of these substances from food and air, it is highly likely that steady state levels are maintained in humans and other animals throughout their lifetime. Clearly, bioaccumulation of these terpene hydrocarbons does not occur, since the substances are efficiently metabolized to yield oxygenated metabolites (*e.g.,* verbenol, myrtenol and myrtenic acid) that are subsequently conjugated with glucuronic acid and excreted mainly in the urine.

3 Test Plan

3.1 Chemical and Physical Properties

3.1.1 Melting Point

Literature values are available for *alpha*-pinene (-55 ^{o}C), camphene (51-2 ^{o}C), and *cis*-pinane (-53 ^{o}C) [CRC Handbook of Chemistry and Physics, 1986]. *beta*-Pinene, a positional isomer of *alpha*-pinene would be expected to have very similar physical properties and the melting point should be on the same order (-55 ^{o}C). Dihydropinene is a mixture of stereoisomers, one of which is *cis*-pinane, and therefore the melting point should be on the same order (-53 ^{o}C). *l-alpha*-Pinene is one stereoisomer of *alpha*-pinene and therefore would have the same melting point (-55 ^{o}C). The four mixtures are all primarily made up of *alpha*- and/or *beta*-pinene and therefore would be expected to have similar but lower melting points.

3.1.2 Boiling Point

Literature values are available for *alpha*-pinene (155-156 ^{o}C @ 760 mm), *beta*-pinene (165-166 ^{o}C @ 760 mm), camphene (158.5-159.5 ^{o}C @ 760 mm), *cis*-pinane (169 ^{o}C @ 760 mm) and dihydropinene (164.5-165 ^{o}C @ 760 mm) [Merck Index, 1996; CRC Handbook of Chemistry and Physics, 1986]. *l-alpha*-Pinene is one stereoisomer of *alpha*-pinene and therefore would have the same boiling point (155-156 ^{o}C @ 760 mm). The four mixtures are all primarily made up of *alpha*- and/or *beta*-pinene and therefore would be expected to have similar but somewhat lower boiling points.

3.1.3 Vapor Pressure

Calculated values [Meylan, 1994-1995c] are available and are in the range from 0.24 – 0.55 kPa at 25 ^{o}C (1.8-4.1 mm Hg) for *alpha*-and *beta*-pinene, *cis*-pinane, dihydropinene, and camphene. Experimental data exists for *cis*-pinane and camphene. The experimentally determined vapor pressure of *l-alpha*-pinene is reported to be 0.53 kPa (3.95 mm Hg) at 25 ^{o}C (Fichon, *et al*., 1999). In an experiment measuring vapor pressure from 441 to 421 ^{o}K, cis-pinane exhibits a vapor pressure of 0.73 kPa (5.47 mm Hg) at 25 ^{o}C (Zhu *et a*l., 2003). The experimental and calculated vapor pressure for pinane and pinene ate in good agreement.

The vapor pressure for camphene is reported to be 3.8 kPa (28.5 mm Hg) for at 20 ^{o}C [Hoechst AG, 1991b]. The calculated value for camphene is based on measured values of 40 kPa and 901.1 kPa for camphene at 62.1 and 154.3 ^{o}C, respectively [Hoechst AG, 1991b]. In a second experiment for camphene, the vapor pressure was reported to be 2.4 kPa (21.1 mm Hg) [Hoechst AG, 1990]. The range of vapor pressure at ambient temperature is fairly narrow. The measured vapor pressure range of 0.5-3.8 kPa is approximately an slightly greater than the calculated values (0.24-0.55 kPa).

3.1.4 n-Octanol/Water Partition Coefficient

There are two reports of measured log Kow values for *alpha*-pinene, the most reliable, 4.83, being from the Syracuse Research Corporation database [Li and Perdue, 1995], which compares favorably with the calculated value of 4.27 [Meylan, 1993-1995a]. The other study, which followed OECD Guideline 117, used a sample of *alpha*-pinene that also contained *beta*-pinene, *delta*-3-carene and camphene. In this study, three unidentified components of *alpha*-pinene at pH 7.5 had log Pow values above 1.5 and were reported as follows, 5.3, 5.5 and 5.7 [Dybdahl, 1993a]. A measured value is also available for the structurally related mixture containing mainly *delta*-3-carene, by the same authors. 3-Carene was reported to consist mainly of *delta*-3-carene, *alpha*-pinene, *beta*-pinene, and dipentene, but the authors provided no analytical data. The log Pow

values were reported as follows for four unidentified components of 3-carene at pH 7.5: 4.6, 5.2, 5.3 and 5.5 [Dybdahl, 1993b].

For the remainder of the materials, only calculated values are available and these range from 4.35 to 4.83. The narrow range and the close agreements with the one measured value in this group indicate consistency and imply reliability. Based on the mutual agreement of measured and calculated values, no further partition coefficient studies are recommended.

3.1.5 Water Solubility

The reported water solubilities for *alpha*-pinene and *beta*-pinene are 0.65 and 2.1 mg/L at 25 °C respectively [Broderius *et al*., 1990] while the calculated values [Meylan, 1993-1995b] are 1.9 and 4.9 mg/L. The measured solubility of camphene (4.2 mg/L at 20 ^{o}C) is in the same range as for pinene [Hoechst AG, 1991c]. The close agreement between calculated and measured values gives confidence in the model used for this group. The solubility of all members of this chemical category at 25 ^{o}C is expected to be in the range from 0.50 to 6.0 mg/L. No further solubility studies are recommended.

3.1.6 New Testing Required

No additional testing is recommended.

3.2 Environmental Fate and Pathways

3.2.1 Photodegradation

The calculated photodegradation half-lives [Meylan, 1994-1995b] for the structurally defined materials in this group are in the range from 1.4 to 9.4 hours. These calculations are based on measured OH rate constants for *alpha*-pinene, *beta*-pinene, camphene and *trans*-pinane, measured ozone and NO_3 rate constants with the exception of *trans*-pinane [AOPWIN]. Therefore, these figures can be considered reliable.

3.2.2 Stability In Water

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

3.2.3 Biodegradation

Five GLP experimental studies evaluating biodegradability are available for this group of substances using standard OECD Guideline protocols. Additional studies in soil horizons obtained from coniferous and deciduous forests [Misra *et al.*, 1996] provide a broader perspective on the biodegradation of bicyclic terpene hydrocarbons in the environment. In standard OECD guideline studies, these terpene hydrocarbons are not readily biodegradable. However, terpene hydrocarbon isomers such as limonene are know to biodegrade in upper soil horizons within 1 hour [Misra *et al.*, 1996]. Since bicyclic terpene hydrocarbons are ubiquitous in the environment, pinene and pinane members of this category are not persistent and do ultimately biodegrade in the environment.

Four studies on *alpha*-pinene showed limited biodegradability. The first, which followed OECD Guideline 302C and evaluated inherent biodegradability, reported 37% biodegradation at 31 days [Rudio, 1999a]; the second, which followed OECD Guideline

301F and evaluated ready biodegradability, reported 38% biodegradation at 28 days [Rudio, 1999b]; and a third, which followed OECD Guideline 301D and evaluated ready biodegradability using a mixture mainly of *alpha* and *beta*-pinene in a closed bottle test, reported very limited biodegradability [Madsen, 1993a]. In the fourth experiment, a mixture of 50.9% *alpha*-pinene and 36.8% *beta*-pinene was concluded to be inherently biodegradable based on the results of a closed bottle Sturm test. The mixture was 52% biodegraded within 28 days, but there was no indication that biodegradation had ceased (Long, 2001b).

Very limited biodegradability was also reported for 3-carene using an OECD Guideline 301D test protocol [Madsen, 1993b] and for camphene (less than 20%) using a standard OECD Guideline test protocol [Hoechst AG, 1988a]. In studies showing limited biodegradability, the authors concluded that the high vapor pressure and low water solubility of these substances led to volatilization of the test substance in the upper parts of the test vessel, thereby, limiting aerobic biodegradation [Rudio, 1999a; 1999b]. Calculated values by BIOWIN [Meylan, 1994a] give the same results for all of the structurally related materials, *i.e.* calculation by the linear model predicts the substances to be on the borderline between fast and slow degradation while the non-linear model predicts they will not biodegrade fast. Given the results of the experimental tests using *alpha*-pinene and *delta*-3-carene, the non-linear model appears to be the more appropriate model for this group.

Additional studies in extracts and slurries prepared from soils of coniferous and deciduous forest indicate rapid and complete biodegradation of *alpha*-pinene 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 *alpha*-pinene for 24 hours. *alpha*-Pinene underwent 100% biodegradation after approximately 8 days in acclimated medium and after day 15 in non-acclimated medium. The authors concluded the pinene is completely degradable in extracts prepared from watershed soils of coniferous or deciduous forests [Misra *et al.*, 1996].

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.

3.2.5 New Testing Required

No additional testing is recommended.

3.3 Ecotoxicity

3.3.1 Acute Toxicity to Fish

The three principal substances in this group, *alpha*-pinene, *beta*-pinene, and camphene have measured fish acute toxicities [Broderius *et al*., 1990]. The acute fish toxicity of a mixture of *alpha*-pinene and *beta*-pinene has also been assessed (Swarbrick, 2001).

The closeness of the 96-hour LC50 measured values for *alpha*-pinene and *beta*-pinene, 0.28 and 0.5 mg/L in fathead minnows, respectively, and the agreement of these measured values with the ECOSAR calculated ones [Nabholz and Cash, 1998], 0.22 and 0.62 mg/L, respectively, effectively validates the ECOSAR model. In a closed system flow-through test with *Brachydanio rerio* following OECD Guideline 203, camphene exhibited a 96-hour LC50 of 0.72 mg/L [Hoechst AG, 1993]. The calculated values for camphene, *cis*-pinane, dihydropinene, and *l-alpha*-Pinene, are 0.62, 0.63, 0.63 and 0.28 mg/L, respectively. These values indicate that all of these materials and the four mixtures that are made up primarily of these substances, should have acute fish toxicities on the order of 0.5 mg/L. The acute toxicity of a mixture of 50.9% *alpha*-pinene and 36.8% *beta*-pinene (gum turpentine) has been evaluated in rainbow trout in a modified OECD 203 Guideline test (Swarbrick, 2001). Although an 96-hour NOEC of greater than 100 mg/L was reported, the experiment was performed using water accommodated fractions in which solutions of gum turpentine were prepared by stirring appropriate weight of test substance for 23 hours followed by 1 hour settling time prior to fish being introduced. Therefore, the actual test concentrations are not known, but may assumed to be at the limit of solubility at 15^{o} C. No deaths were observed at any nominal concentration tested [Swarbrick, 2001].

3.3.2 Acute Toxicity to Aquatic Invertebrates

The two principal substances in this group, *alpha*-pinene and *beta*-pinene have measured *Daphnia magna* acute toxicities. The closeness of these 48-hour LC50 measured values, 1.44 and 1.25 mg/L [Broderius *et al*., 1990], respectively, and the agreement of these measured values with the ECOSAR 48-hour LC50 calculated ones [Nabholz and Cash, 1998], 0.22 and 0.79 mg/L, respectively, effectively validates the conservative nature of the ECOSAR model. Forty-eight hour calculated LC50 values are typically lower by an order of magnitude than experimental values.

The 48-hour LC50 values for *Daphnia magna* using pinene in lake water in a semistatic OECD Guideline 202 [Bjornestad, 1993b] and for camphene [Hoechst AG, 1980] are higher than the limit of solubility for these two substances. Therefore, these results are considered not reliable. Similarly, in an OECD Guideline 202 test, the 48-hour EC50 value for a mixture of 50.9% *alpha*-pinene and 36.8% *beta*-pinene (gum turpentine) was reported to be 10 to 100 mg/L with an NOEC value of 10 mg/L (Long, 2001a). However, this experiment was carried out with water-accommodated fractions that exceeded the limits of solubility of the test substance. However, it may be safely assumed that camphene and gum turpentine are not acutely toxic to *Daphnia magna* at their solubility limits. The calculated values for camphene, *cis*-pinane, dihydropinene, and *l-alpha*-pinene, 0.79, 0.8, 0.8 and 0.22 mg/L, respectively, indicates that all of these materials and the four mixtures that primarily are made up of these substances, should all have acute aquatic invertebrate toxicities on the order of 1.0 mg/L.

3.3.3 Acute Toxicity to Aquatic Plants

The two principal substances in this group, *alpha*-pinene and *beta*-pinene have measured algae acute toxicities [Broderius *et al*., 1990]. The 48-hour EC50 is greater than the solubility (measured as 0.65 mg/L) for *alpha*-pinene, and for *beta*-pinene it is 1.44 mg/L. The agreement of these values with the ECOSAR calculated values [Nabholz and Cash, 1998], 0.22 and 0.79 mg/L, respectively, effectively validate the ECOSAR model. The

reported value of 72-hour EC50 of 1000 mg/L reported for camphene is not reliable given it far exceeds the measured solubility (4.2 mg/L) of camphene [Hoechst AG, 1991d]. Similarly, the algal (*Selenastrum capricornutum*) 72-hour EC50 value of greater than 100 mg/L (Long, 2000) exceeds the limit of solubility for a mixture 50.9% *alpha*-pinene and 36.8% *beta*-pinene (gum turpentine). However, it may be safely assumed that camphene and gum turpentine are not acutely toxic to *Daphnia magna* at their solubility limits. The 96-hour calculated values for camphene, *cis*-pinane, dihydropinene, and *l-alpha*-pinene, 0.56, 0.57, 0.57 and 0.22 mg/L, respectively, indicates that all of these materials and the four mixtures that primarily are made up of these substances, should all have acute aquatic plant toxicity on the order of 0.5 mg/L.

3.3.4 New Testing Required

No additional testing is recommended.

3.4 Human Health Toxicity

Results of available acute toxicity tests for single exposure *via* the oral, dermal, and inhalation route provide a baseline of data to interpret the consequences of single high dose exposures occurring from intentional or unintentional poisonings or occupational accidents. The literature abounds with clinical reports of accidental and intentional acute poisoning with pinene-based turpentine [*e.g.*, Koppel *et al.,* 1981].

The requirements for further testing human health toxicity endpoints that evaluate chronic exposure must be considered in the context of the unavoidable, continuous exposure of humans to bicyclic terpenoid hydrocarbons. Humans have evolved in a biosphere in which *alpha*-pinene and other structurally related terpene hydrocarbons are ubiquitous. Conservative estimates of combined daily intake from consumption of foods and inhalation of air are in the range from 10-30 mg. For many individuals intakes may exceed 100 mg/day. Of this total exposure greater than 95% of oral intake is derived from consumption of traditional foods while greater than 98% of inhalation exposure is derived from biogenic emissions from plants. The contribution of anthropogenic sources to chronic exposure in humans is insignificant. Therefore, human health risk to *alpha*-pinene and other bicyclic terpene hydrocarbons is unavoidable. In the absence of any significant increase in the anthropogenic emissions, additional studies of repeat-dose toxicity, reproductive and developmental toxicity, and assays screening for genotoxic effects from long-term exposure seem unnecessary.

3.4.1 Acute Toxicity

Rat oral LD50 values are available for *alpha*-pinene, *beta*-pinene, camphene and turpentine oil and indicate these materials to be very low in oral acute toxicity with LD50 values in the range from 3388 mg/kg to greater than 5000 mg/kg [Moreno, 1972a, 1974a, 1975a; Piccirillo, 1984; von Skramlik, 1959]. Rabbit dermal LD50 values similarly indicate very low toxicities with values greater than the limit doses of 2000 or 5000 mg/kg [Moreno, 1972b, 1972c, 1974b, 1975b]. The remaining materials are expected to

have similar values. Acute inhalation toxicity has been measure in different animal species. The acute LC50 was reported to be 13,500 mg/m^3 in rats, 13,500 mg/m^3 in guinea pigs, and 9000 mg/m^3 in mice [Kohn, 1962]. The acute inhalation LC50 of commercial grade turpentine in Wistar rats is reported to be in the range of 12,000-20,000 mg/m^3 for 1 to 6 hour exposures and the LC50 for a 2-hour exposure in Swiss-Webster mice is 29,000 mg/m^3 [Sperling *et al.*, 1967]. Based on these results the acute oral, dermal, and inhalation toxicities of bicyclic terpene hydrocarbons is concluded to be low.

3.4.2 Genetic Toxicity

3.4.2.1 *In vitro* Genotoxicity

In vitro genotoxicity assays available for *alpha*-pinene, *beta*-pinene and camphene demonstrate that these substances have a little, if any, genotoxic potential. In standard Ames assays of *alpha*-pinene, *beta*-pinene and camphene, *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, TA1537, and TA1538 provided no evidence of mutagenicity at any dose tested [Rockwell and Raw, 1979; Florin *et al*., 1980; Heck *et al*., 1989; Jagannath, 1984; DeGraff, 1983; Connor *et al.,* 1985]. Recently, the (+)- and (-)-alpha-pinene has been tested in *Salmonella typhimurium* strains TA97a, TA98, TA100, and TA1535 with and without s-9 activation (Gomes-Carneiro *et al*., 2005). There was no evidence of mutagenicity at concentrations up to 1000 ug/plate for the (+) form and 4000 ug/plate for (-)-*alpha*-pinene.

In an *in vivo*-*in vitro* study designed to investigate the mutagenicity of the metabolites of *alpha*-pinene and camphene, Sprague-Dawley rats were administered a single dose of 0.5 ml (452 mg) of *alpha*-pinene or camphene by gavage and the urine was collected for 24 hours. To assess the genotoxic potential of urinary metabolites, the urine extract was treated with *beta*-glucuronidase to hydrolyze glucuronic acid conjugates. The substances, the urine samples (500 microliters), the ether extracts of the urine, and the aqueous fractions of the urine-ether extracts were then separately incubated with *Salmonella typhimurium* strains TA98 and TA100 with S9 activation. *alpha*-Pinene, camphene

(tested directly) and the urinary solutions isolated from the rats administered 0.5 ml of *alpha*-pinene did not show any evidence of mutagenicity in either TA98 or TA100 with metabolic activation. Assays of the ether extract of the urine from animals administered 0.5 ml camphene showed a weak response in TA100 with metabolic activation, but both the urine sample directly and the aqueous fraction of the urine-ether extract did not [Rockwell and Raw, 1979].

In a Ames assay with camphene itself, there were no effects on several strains of *Salmonella typhimurium*, including TA100, with or without activation [Connor *et al*., 1985]. Similarly, *alpha*-pinene did not induce unscheduled DNA synthesis in rat hepatocytes [Heck *et al*., 1989] and neither *beta*-pinene nor camphene gave any evidence of sister chromatid exchange in cultured Chinese hamster ovary cells [Sasaki *et al.*, 1989]. Therefore, all members of this chemical category are expected to show no significant genotoxic potential *in vitro*.

3.4.2.2 *In vivo* Genotoxicity

An *in vivo* mouse micronucleus assay using an OECD Guideline 474 protocol has been performed with camphene [Hoechst AG, 1991e]. In this study, groups of male and female (5/sex/group) were given a single oral dose of 0 or 4000 mg/kg bw of camphene by gavage. There was no increase in the incidence of micronucleated polychromatic erythrocytes in the treatment group compared to controls. Based on the results of this *in vivo* genotoxicity assay and the lack of any evidence of genotoxicity for numerous *in vitro* assays with and without metabolic activation, it is unlikely that any of these materials would exhibit a significant genotoxic potential *in vivo*.

Based on the uniformly negative results for members of this category, no additional *in vitro* and *in vivo* assays are recommended.

3.4.3 Repeat Dose Toxicity

alpha-Pinene has been subjected to 90-day inhalation study in mice and rats (NTP, 2006a). In the rat study, groups of F344/N rats (10/sex/group) were exposed to atmospheres of 0, 25, 50, 100, 200, or 400 ppm of α-pinene for 6 h/day, 5 days per week for 14 weeks. Based on an absorption rate of 50%, these inhalation concentrations correspond to estimated daily intakes of 0, 21, 42, 85, 170 or 340 mg/kg bw per day (Fasset, 1978). The animals were observed twice per day and weighed once per week. A complete histopathologic evaluation inclusive of treatment-related gross lesions were performed on all early death animals regardless of dose group, all control animals, all animals, and all animals in the highest treatment group with at least 60% survivors at the time of sacrifice plus all animals in higher treatment groups. Treatment-related lesions (target organs) were identified and these organs plus gross lesions were examined to a no-effect level.

All of the exposed males showed a decrease in body weight gain when compared to controls while the females exposed to less than 200 ppm showed a slight increase in body weight gain when compared to controls. Six female rats of the 400 ppm group were found dead during the study and 3 female rats of the same high exposure group displayed mild tremors. Absolute and relative liver weights were statistically increased in males at 200 ppm and greater and relative and absolute kidney weights were increased in males at 100 ppm and greater. In females, relative and absolute liver weights were increased at levels of ≥50 ppm, but there were no increases in either hepatic enzymes or any evidence of histopathological changes at any of these dose levels. Females showed statistically significant decreases in absolute and relative thymus weights and increased relative lung weight at the 400 ppm level.

Males showed statistically significant reductions in sorbitol dehydrogenase activity at 400 ppm, alanine aminotransferase activity at levels ≥50 ppm, and alkaline phosphatase activity at levels ≥100 ppm. Females showed statistically significant reductions in alanine aminotransferase activity at levels ≥200 ppm, and alkaline phosphatase activity at the 400 ppm. There were significant decreases at lower levels of exposure for females but these

changes were not dose-dependent. None of these changes in enzyme activity were related to organ weight changes or evidence of histopathology. Examination of the male kidneys at all dose levels revealed lesions including granular casts and hyaline droplets typical of α_{2u}-globulin nephropathy. It has been concluded that α_{2u}-globulin nephropathy is specific to the male rat and is not relevant to human health assessments (EPA, 1990). In females, there was no evidence of histopathology in any organ at any dose level. There was no evidence of histopathological changes to the clitoris, ovaries, uterus, epididymis, preputial gland, seminal vesicles, and testes any of the control or test groups of animals. Based on these observations, the NOAEL for male rats was 25 ppm or 21 mg/kg bw per day and the NOAEL for female rats was 200 ppm or 170 mg/kg bw per day.

In the mouse study, B6C3F1 mice (10/sex/group) were exposed to atmospheres of 0, 25, 50, 100, 200, or 400 ppm of α-pinene for 6 h/day, 5 days per week for 14 weeks (NTP, 2006b draft). Based on an absorption rate of 50%, these inhalation concentrations correspond to estimated daily intakes of 0, 36, 72, 144, 288, and 576 mg/kg bw per day (Fasset, 1978). The study protocol for mice was the same as for the 14-week rat study.

All mice survived until the study was completed. Body weight gain was comparable for all test animals when compared to controls. Absolute liver weights were increased for both sexes at the 400 ppm and relative and absolute liver weights were increased for both sexes at 200 ppm and 400. The 400 ppm male group showed decreased absolute and relative thymus weight. No gross or microscopic lesions were associated with these organ weight findings.

Histopathological examination of male and female mice exposed to atmospheres of ≥100 ppm of α-pinene revealed evidence of hyperplasia of the transitional epithelium of the urinary bladder. However, there was no evidence of histopathological changes to the clitoris, ovaries, uterus, epididymis, preputial gland, seminal vesicles, and testes any of the control or test groups of animals. Based on these observations, a NOAEL for both male and female mice was concluded to be 50 ppm. This dose is estimated to be approximately equal to 72 mg/kg bw per day for both male and female mice.

A 28-day repeat dose study has been performed with camphene according to an OECD Guideline 407 in both sexes of Wistar rats [Hoechst, 1991f]. Groups of animals (5/sex/group) were given daily oral doses of 0, 62.5, 250, or 1000 mg/kg bw by gavage for 28 days. Weekly measurement of body weight and food intake revealed no significant differences between test and control animals. Animals of both sexes at the 1000 mg/kg bw/day dose exhibited vacuolization of hepatocytes and increase liver weights. Male rats also exhibited *alpha*-2-microglobulin-type nephrotoxicity at all dose levels.

The renal pathology reported in F344N male rats is a sex- and species-specific phenomenon. The following interpretation of the nephrotoxicity reported in the 28-day study with camphene is based on recent key studies referenced below. No robust summaries have been prepared for these studies because they investigate the various aspects for the mechanism of action of *alpha*-2-microglobulin-induced nephrotoxicity.

It is now well recognized that renal lesions observed in above study, resulted from the accumulation of aggregates of *alpha*-2-microglobulin (a low molecular-weight protein synthesized in the liver) and camphene 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 *et al*., 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 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 does not develop in mammals that do not express the hepatic form of *alpha*-2-microglobulin [Swenberg *et al.,* 1989] such as other strains of rats [Dietrich and Swenberg, 1991], mice [Bucher *et al*., 1986; Lehman-McKeeman and Caudill, 1994] and dogs [Webb *et al*., 1990]. Therefore, the nephrotoxicity observed in the camphene study in male F344 rats is not relevant to the human health risk assessment. Based on liver toxicity, the NOAEL for this study is concluded to be 250 mg/kg bw/day.

Several repeat dose inhalation studies on the related substance turpentine are available. These studies were performed using Beagle dogs, Sprague-Dawley rats, English strain guinea pigs, Long-Evans hooded rats and Swiss white mice exposed to 2.4 or 4.8 mg/L turpentine vapor for six hours each day, five days per week for 30-84 days [Kay, 1963; Calandra, 1964]. Some toxic effects were reported, but are hard to evaluate given the lack of controls, and single dose design. These results are not considered reliable.

The principal *in vivo* metabolite of *alpha*-pinene is verbenone which is excreted as verbenol. A 28-OECD guideline study has been performed with verbenone (Jones, 2003). Groups of male and female Sprague-Dawley rats were administered verbenone via gavage for twenty-eight consecutive days, at a single dose level of 10 mg/kg/day. A control group of ten males and ten females was dosed with vehicle alone. Clinical signs, bodyweight development and food and water consumption were monitored throughout the study. Haematology and blood chemistry were evaluated for all animals at the end of the study. At study termination, gross necropsies were performed on all of the animals. Histopathological evaluations were conducted on selected tissues from all of the animals. No clinically observable signs of toxicity were reported. There were no adverse effects on body weight, survival, food consumption, water consumption, haematological or blood chemistry parameters. Organ weights for the test animals were comparable to controls. No treatment-related macroscopic effects were reported. Histopathological examination revealed globular accumulations of eosinophilic material in the tubular epithelium of male rats treated at 10 mg/kg bw per day. This finding is consistent with the presence of hydrocarbon nephropathy, which results from the excessive accumulation of α-2u-globulin in renal proximal tubular epithelial cells. α-2u-globulin is found only in the proximal tubular epithelium of adult male rats (IARC, 1999). There was no toxicologically significant difference in incidence or distribution of severity grades of this condition between animals administered verbenone, or nootkatone, a structurally related ketone, which was administered in a parallel study. Oral administration of verbenone to rats for a period of twenty-eight consecutive days at a single dose level of 10 mg/kg/day did not result in any toxicologically significant effects.

A 90-day repeat-dose toxicity test is available for polyterpene, which is a resin of *beta*-pinene. The test material was not analyzed for the presence of the monomer, *beta*-pinene. Therefore, the study must also be regarded as unreliable. Five groups of 20 Sprague-Dawley male and female albino rats each were administered in corn oil 0.01, 0.05, 0.2, 1.0 and 5.0% polyterpene, corresponding to 5.82, 29.58, 116.5, 586.2 or 2788.7 mg/kg bw/d, in the diet for 90 days. Observations included growth, food consumption, mortality, and status of hematopoietic and urinary systems. All animals were sacrificed at the conclusion of the study, and necropsies were performed on all animals. Histopathological examinations were conducted on selected animals from the control and test groups. No differences were seen between the test and control animals for the following parameters: growth, food consumption and utilization, mortality, hematologic and urine analyses, gross pathologic findings and histopathological findings. Elevated liver weights were reported for the two highest-level treatment groups. One male from the 0.05% and one male from the 1.0% test groups died during the study. These deaths were attributed to respiratory illness. Statistically significant differences in liver weights were reported for 1% and 5% treatment groups. Histopathological examination revealed no differences between test and control animals. Under the conditions of this study, the NOAEL is considered to be 116.5 mg polyterpene/kg bw/day [Calandra, 1962].

Fourteen-week inhalation toxicity study performed with *alpha*-pinene in both mice and rats showed NOAELs of 72 mg/kg bw per day in both sexes of mice and 170 mg/kg bw per day for female rats. Given the presence of *alpha*-2-microglobulin nephrotoxicity in male rats dosed with *alph*a-pinene, male rats are not a valid model for performing a human health hazard assessment. Similarly, the camphene study result must be evaluated in the context of species and sex specific *alpha*-2-microglobulin nephrotoxicity. Therefore, the nephrotoxicity observed in the camphene study in male F344 rats is not relevant to the human health risk assessment. Based on liver toxicity in this study at 1000 mg/kg bw per day, the NOAEL for this study is concluded to be 250 mg/kg bw/day. *alpha*-Pinene and camphene exhibit a pattern of toxicity and genotoxicity typical of monoterpene hydrocarbons (see Test Pan and Robust Summaries for Monoterpene Hydrocarbons). The target organ effects and levels of toxicity from exposure to monoterpene hydrocarbons such as limonene and myrcene are super imposable with

those of *alpha*-pinene and camphene. Clearly, the sensitivity of current standardized toxicologic assays indicate that aliphatic branched-chain (myrcene), monocyclic (limonene), and bicyclic (pinene and camphene) hydrocarbons exhibit nearly equivalent toxic potential. Uniformly negative results of both *in vitro* and *vivo* genotoxicity assays support this conclusion.

3.4.4 Reproductive Toxicity

In the 14-week inhalation study in both mice and rats (NTP, 2006), there was no evidence of histopathological changes to the clitoris, ovaries, uterus, epididymis, preputial gland, seminal vesicles, and testes in any of the control or test animals receiving doses up to 400 ppm (up to 340 mg/kg bw per day in rats and 576 mg/kg bw per day in mice). When this data is combined with the fact that no adverse effects were observed to the reproductive organs in a 28-day developmental study with camphene [Hoechst AG, 1991f] at dose levels up to 250 mg/kg bw/day, it is concluded that bicyclic terpene hydrocarbons including *alpha*-pinene and *beta*-pinene exhibit no potential for reproductive toxicity. Additionally, an FDA study (see below) including evaluation of many parameters monitored in a standard reproductive toxicity study showed no evidence to conclude that these substances provided any significant reproductive hazard.

A structurally related C_{10} terpene hydrocarbon beta myrcene has been the subject of an reproductive study in male and female Wistar rats. Three experimental groups (15 male and 45 female rats per group) were administered *beta*-myrcene (CAS No. 123-35-3) 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].

In a Food and Drug Administration-sponsored study [Morgareidge, 1973a, 1973b, 1973c] that evaluated both reproductive and developmental toxicity parameters, an essential oil consisting predominantly (80-90%) of bicyclic terpene $C_{10}H_{16}$ hydrocarbons {*alpha*-pinene (20-25%), *beta*-pinene (15-18%) and sabinene (38-42%)} was given to pregnant CD-1 mice, Wistar rats, or golden hamsters. In the mouse study, groups (20-21/group) of pregnant female CD-1 outbred mice were given 0, 6, 26, 120, or 560 mg/kg bw of the test

material (FDA 71-28) by gavage in corn oil on days 6-15 of gestation. A positive control group received 150 mg/kg bw/day of aspirin. Maternal body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On Day 17 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects.

The administration of up to and including 560 mg/kg bw/day of test article FDA 71-28 to pregnant mice on days 6 through 15 of gestation had no effects on nidation, reproduction, maternal survival or any measured fetal parameter. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls.

The rat and hamster studies use the same study protocol. Adult female Wistar or golden hamsters were individually housed in mess-bottom cages in a temperature- and humidity-controlled room. There were mated with untreated young adult males and observation of vaginal sperm plugs (rats) or appearance of motile sperm in vaginal smears (hamsters) was considered day 0 of gestation. Groups (22-23/dose) of pregnant Wistar rats were then given 0, 3, 2, 56, or 260 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil daily on Day 6 and through Day 15 of gestation [Morgareidge, 1973c]. Groups (26-28/dose) of pregnant hamsters were given 0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil daily on Day 6 and through Day 10 of gestation [Morgareidge, 1973b]. In the rats or hamster study, a positive control group received 250 mg/kg bw/day of aspirin.

The administration of up to and including 260 mg/kg bw/day of test article FDA 71-28 to pregnant rats on days 6 through 15 of gestation or administration of up to and including 600 mg/kg bw/day to pregnant golden hamsters on Day 6 through 10 of gestation had no effects on nidation, reproduction, maternal survival or any measured fetal parameter.

In the three-species study, no reproductive effects were observed when dose levels of up to 260 to 600 mg/kg bw of an essential oil predominantly composed of bicyclic terpene hydrocarbons (*alpha*-pinene, *beta*-pinene, and sabinene) was administered daily to mice, rats, or hamsters during gestation.

Based on the lack of effects to reproductive organs in both male and female F344/n rats and B6C3F1 mice in 90-day toxicity studies (NTP, 2006), the NOAEL of 300 mg/kg bw/d for reproductive effects in male and female Wistar rats (Paumgartten *et al.*, 1998), and the lack of any effects to reproductive organs in a multiple species study using an oil containing >80% terpene hydrocarbons, mainly pinene and sabinene (Morgareidge, 1973a,b,c), it can be concluded that bicyclic monoterpene hydrocarbons exhibit a low order of reproductive toxicity.

3.4.5 Developmental/Teratogenicity Toxicity

A developmental screening test is available on a commercial mixture containing *alpha*- and *beta*-pinene (17%) and camphene (5%) [Hasegawa and Toda, 1978]. In this study, a maternal and fetal NOAEL for the mixture was determined to be 0.8 ml/kg (688 mg/kg bw) while mild signs of toxicity in terms of decreased bodyweights were seen at 1.6 ml/kg (860 mg/kg bw). There were no gross, visceral or skeletal anomalies seen at the highest dose level. This could be considered a screening test for *alpha*- and *beta*-pinene at 17% of the daily dose and for camphene at 5% of the daily dose.

In a developmental study performed according to an OECD Guideline 414, Sprague-Dawley rats were given daily oral doses of 0, 250, or 1000 mg/kg bw/ day of camphene on days 6-15 of gestation [Hoechst AG, 1992]. Temporary clinical symptoms in dams at the 1000 mg/kg bw level included reduced motor activity and salivation on days 1 and 2

of treatment. No teratogenic effects were reported in any offspring. The maternal and developmental NOAEL were reported to be 250 and 1000 mg/kg bw/day, respectively.

In the FDA sponsored study discussed above [Morgareidge, 1973a, 1973b, 1973c], female pregnant CD-1 mice, Wistar rats, and golden hamsters were given dose levels of up 560, 260, and 600 mg/kg bw, respectively, of an essential oil containing > 80% bicyclic terpene hydrocarbons daily by gavage during gestation. Based on clinical observations and measurement of body weight gain, mortality, and evaluation of the urogenital tract of pregnant females there were no signs of maternal toxicity at any dose level in any of the three species. Based on measurement of fetal survival, fetal body weight, visceral examination of pups, and a complete skeletal examination of pups at all dose levels, there was no evidence of developmental toxicity at any dose level in any of the three species.

Based on the NOAELs for maternal and developmental toxicity in studies with camphene (250 and 1000 mg/kg bw/day) [Hoechst AG, 1992] and a terpene hydrocarbon mixture containing *alpha*- and *beta*-pinene and camphene (688 mg/kg bw/day) [Hasegawa and Toda, 1978], and the lack of any signs of maternal or developmental toxicity in a mice, rats, or hamsters given 260 to 600 mg/kg bw/day of a mixture composed primarily (>80%) of *alpha*- and *beta*-pinene and sabinene [Morgareidge, 1973a, 1973b, 1973c], it is concluded that bicyclic terpene hydrocarbons are not maternal or developmental toxicants.

3.4.6 New Testing Required

No additional testing recommended.

3.5 Test Plan Table

Chemical	Chemical and Physical Properties				
	Melting Point	**Boiling Point**	**Vapor Pressure**	**n-Octanol/Water Partition Coefficient**	**Water Solubility**
CAS No. 80-56-8 *alpha*-Pinene	A	A	A, Calc	A, Calc, R	A, Calc
CAS No. 127-91-3 *beta*-Pinene	A	A	A,Calc	Calc	A, Calc
CAS No. 79-92-5 Camphene	A	A	A, Calc	Calc	A, Calc
CAS No. 6876-13-7 *cis*-Pinane	A	A	Calc	Calc	Calc
CAS No. 473-55-2 Dihydropinene	A	A	Calc	Calc	Calc
CAS No. 7785-26-4 *l-alpha*-Pinene	A	A	Calc	Calc	Calc
CAS No. 65996-96-5 Terpenes & Terpenoids, Turpentine oil, *alpha*-Pinene fraction	R	R	Calc	Calc	Calc
CAS No. 65996-97-6 Terpenes & Terpenoids, Turpentine oil, *beta*-Pinene fraction	R	R	Calc	Calc	Calc
CAS No. 9005-90-7 Turpentine gum	R	R	Calc	Calc	Calc
CAS No. 8006-64-2 Turpentine oil	R	R	Calc	Calc	Calc

Chemical	Environmental Fate and Pathways			
	Photodegradation	**Stability in Water**	**Biodegradation**	**Fugacity**
CAS No. 80-56-8 *alpha*-Pinene	Calc	NA	A, Calc, R	Calc
CAS No. 127-91-3 *beta*-Pinene	Calc	NA	Calc	Calc
CAS No. 79-92-5 Camphene	Calc	NA	A, Calc	Calc
CAS No. 6876-13-7 *cis*-Pinane	Calc	NA	Calc	Calc
CAS No. 473-55-2 Dihydropinene	Calc	NA	Calc	Calc
CAS No. 7785-26-4 *l-alpha*-Pinene	Calc	NA	Calc	Calc
CAS No. 65996-96-5 Terpenes & Terpenoids, Turpentine oil, *alpha*-Pinene fraction	Calc	NA	Calc	Calc
CAS No. 65996-97-6 Terpenes & Terpenoids, Turpentine oil, *beta*-Pinene fraction	Calc	NA	Calc	Calc
CAS No. 9005-90-7 Turpentine gum	Calc	NA	A, Calc	Calc
CAS No. 8006-64-2 Turpentine oil	Calc	NA	Calc	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	**Acute Toxicity to Aquatic Invertebrates**	**Acute Toxicity to Aquatic Plants**
CAS No. 80-56-8 *alpha*-Pinene	A, Calc	A, Calc	A, Calc
CAS No. 127-91-3 *beta*-Pinene	A, Calc	A, Calc	A, Calc
CAS No. 79-92-5 Camphene	A, Calc	A, Calc	A, Calc
CAS No. 6876-13-7 *cis*-Pinane	Calc	Calc	Calc
CAS No. 473-55-2 Dihydropinene	Calc	Calc	Calc
CAS No. 7785-26-4 *l-alpha*-Pinene	Calc	Calc	Calc
CAS No. 65996-96-5 Terpenes & Terpenoids, Turpentine oil, *alpha*-Pinene fraction	Calc	Calc	Calc
CAS No. 65996-97-6 Terpenes & Terpenoids, Turpentine oil, *beta*-Pinene fraction	Calc	Calc	Calc
CAS No. 9005-90-7 Turpentine gum	A, Calc	A, Calc	A, Calc
CAS No. 8006-64-2 Turpentine oil	Calc	Calc	Calc

Chemical	Human Health Toxicity					
	Acute Toxicity	**Genetic Toxicity *In Vitro***	**Genetic Toxicity *In Vivo***	**Repeat Dose Toxicity**	**Repro-ductive Toxicity**	**Develop-mental Toxicity**
CAS No. 80-56-8 *alpha*-Pinene	A	A	R	A	A, R	A
CAS No. 127-91-3 *beta*-Pinene	A	A	R	A	R	A
CAS No. 79-92-5 Camphene	A	A	A	A	A, R	A
CAS No. 6876-13-7 *cis*-Pinane	R	R	R	R	R	R
CAS No. 473-55-2 Dihydropinene	R	R	R	R	R	R
CAS No. 7785-26-4 *l-alpha*-Pinene	R	R	R	R	R	R
CAS No. 65996-96-5 Terpenes & Terpenoids, Turpentine oil, *alpha*-Pinene fraction	R	R	R	R	R	R
CAS No. 65996-97-6 Terpenes & Terpenoids, Turpentine oil, *beta*-Pinene fraction	R	R	R	R	R	R
CAS No. 9005-90-7 Turpentine gum	R	R	R	R	R	R
CAS No. 8006-64-2 Turpentine oil	A	R	R	R	R	R

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	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

4 References for Test Plan and Robust Summaries

American Conference of Governmental Industrial Hygienists (ACGHI) (1993-1994) Threshold limit values for chemical substances and physical agents and biological exposure indices, pp.12-35.

Arizona Chemical (1999) Typical analyses of representative terpene-based products for 1999. Private communication to FFHPVC. Unpublished report.

Ashley D.L. and J.D. Prah (1997) Time dependence of blood concentrations during and after exposure to a mixture of volatile organic compounds. *Archives of Environmental Health*, **52(1),** 26-33.

Bauer K. and D. Garbe (1985) Common Flavor and Fragrance Materials, Verlagsgesellschaft mbH, D-6940, Weinheim, Federal Republic of Germany.

Bjornestad E. (1993a) Fish acute toxicity test of *alpha*-pinene with zebrafish. Project 303068, Water Quality Institute, Horsholm, Denmark.

Bjornestad E. (1993b) Immobilization test of *alpha*-pinene with the crustacean *daphnia magna,* Project 303068, Water Quality Institute, Horsholm, Denmark.

Borghoff S.J., B.G, Short, and J.A. Swenberg (1990) Biochemical mechanisms and pathobiology of nephropathy. *Annu. Rev. Pharmacol. Toxicol.,* **30**: 349-367.

Broderius S., Hammermeister D.and C. Russom (1990) Toxicity of eight terpenes to Fathead minnows (*Pimephales promelas*), Daphnids (*Daphnia magna*), and Algae (*Selanastrum capricornutum*). Unpublished report to US EPA.

Bucher J.R., Huff J., and W.M. Kluwe (1986) Toxicology and carcinogenesis studies of isophorone in F344 rats and B6C3F1 mice. Toxicology, **39**(2), 207-219.

Calandra J. C. (1962) Ninety-day subacute oral toxicity of polyterpene - albino rats. Industrial Bio-Test Laboratories, Inc., Unpublished report.

Calandra J. C. (1964) 30-Day subacute vapor inhalation toxicity of turpentine. Industrial Bio-Test Laboratories, Inc., Unpublished report.

CIVO-TNO (1999) *Volatile Components in Food-Qualitative and Quantitative Data*. Supplement 5 to the 6th Edition. Edited by H. Maarse, C.A. Visscher, L.C. Willemsens, L.M. Nijssen, and M.H. Boelens. TNO Nutrition and Food Research. Zeist, The Netherlands.

Connor T.H., Theiss, J., Hanna, H., Monteith, D. and T. Matney (1985) Genotoxicity of organic chemicals frequently found in the air of mobile homes. *Toxicology Letters*, **23**, 33-40.

CRC Handbook of Chemistry and Physics (1986) 67st edition, Robert C. Weast, editor, The Chemical Rubber Co Press, Inc. Boca Raton, FL.

Csiky O. and G. Seufert (1999) Terpenoid emissions of Mediterranean oaks and their relationship to taxonomy. *Ecol. Appl*., **9(4),** 1138-1146.

DeGraff W.G. (1983) Mutagenicity evaluation of *beta*-pinene. Private communication. Unpublished report.

De-Oliveira A.C.A.X., Ribeiro-Pinto L.F. and Paumgartten F.J.R. (1997) *In vitro* inhibition of CYP2B1 monoxygenase by *beta*-myrcene and other monoterpenoid compounds. *Toxicology Letters*, **92(1),** 39-46.

Derfer and Traynor (1992) The Chemistry of Turpentine in "Naval Stores", Editors D. Zinkel and J. Russell.Pulp Chemicals Association. New York, New York.

Dietrich D. R. and J. A. Swenberg (1991) NCI-Black-Reiter (NBR) male rats fail to develop renal disease following exposure to agents that induce *alpha*-2-micro-globulin nephropathy. *Fundamental and Applied Toxicology,* **16**, 749-762.

Dybdahl H.P. (1993a) Determination of log Pow for single components in *alpha*-pinene, Project 303068, Water Quality Institute, Horsholm, Denmark.

Dybdahl H.P. (1993b) Determination of log Pow for single components in 3-Carene, Project 303068, Water Quality Institute, Horsholm, Denmark.

Eriksson K. and J.O. Levin (1990) Identification of *cis*- and *trans*-verbenol in human urine after occupational exposure to terpenes. *International Archives of Occupational and Environmental Health*, **62(5),** 379-383.

Eriksson K. and J.O. Levin (1996) Gas chromatographic mass spectrometric identification of metabolites from *alpha*-pinene in human urine after occupational exposure to sawing fumes. *Journal of Chromatography*, **677(1),** 85-98.

Falk A., Hagberg, M., Lof. A., Wigaeus-Hjelm, E. and W. Zhiping (1990) Uptake, distribution and elimination of *alpha*-pinene in man after exposure by inhalation. *Scand. Journal Work Envirn. Health*, **16(5),** 372-378.

Falk A., Lof, A., Hagberg, M., Hjelm, E., and Z. Wang (1991) Human exposure to 3-carene by inhalation: toxicokinetics, effects on pulmonary function and occurrence of irritative and CNS symptoms. *Toxicology and Applied Pharmacology,* **110**, 198-2055.

Fichan I., Larroche C., and Gros J.B. (1999). Water solubility, vapor pressure, and activity coefficients for terpenes and terpenoids. *J. Chem. Eng. Data*, **44**, 56-62.

Florin I., Rutberg, L., Curvall, M., and C.R. Enzell (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames test. *Toxicology*, **18**, 219-232.

Food and Agriculture Organization (United Nations) (2000) Global Forest Resources Assessment 2000 (FRA 2000) Committee on Forestry Rome Italy 2001. Proceedings of FAO Expert Consultation to Review the FRA 2000 Methodology for Regional and Global Forest Change Assessment. Forest Resources Assessment Programme, Working Paper 42.

Gallagher M., Clayborough, R., Beswick, K., Hewitt, C., Owen, S., Moncrieff, J. and K. Pilegaard (2000) Assessment of a relaxed eddy accumulation for measurements of fluxes of biogenic volatile organic compounds; study over arable crops and a mature beech forest. *Atmos. Environ.*, **34**, 2887-2899.

Gomes-Carneiro M. R., M.E.S.Viana, I.Felzenszwalb and F.J.R.Paumgartten (2005) Evaluation of beta-myrcene, alpha-terpinene and (+)- and (-)-alpha-pinene in the Salmonella/microsome assay. *Food and Chemical Toxicology*, 43(2), 247-252.

Guenther, A., Hewitt, C., Erickson, D., Fall, R., Geron, C., Graedel, T., Harley, P., Klinger, L., and M. Lerdau (1995) A global model of natural volatile organic compound emissions. *J. Geophys. Res, [Atmos.],* **100, D5**, 8873-8892.

Guenther, A., Geron, C., Pierce, T., Lamb, B., Harley, P. and R. Fall (2000) Natural emissions of non-methane volatile organic compounds, carbon monoxide, and oxides of nitrogen from North America. *Atmos. Environ.*, **34**, 2205-2230.

Hall R.L. and Oser, B.L. (1965) Recent progress in the consideration of flavoring ingredients under the food additives amendment. III GRAS Substances. *Food Technology*, **19(2)**, 151-197.

Hasegawa M. and T. Toda (1978) Teratological studies on rowachol. *Oyo Yakuri*, **15(7),** 1109-1119.

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.

Helmig D., Klinger, L., Guenther, A., Vierling, L., Geron, C. and P. Zimmerman (1999a) Biogenic volatile organic compound emissions (BVOCs) I. Identifications from three continental sites in the U.S. *Chemosphere,* **38(9)** 2163-2187.

Helmig D., Klinger, L., Guenther, A., Vierling, L., Geron, C. and P. Zimmerman (1999b) Biogenic volatile organic compound emissions (BVOCs) II. Landscape flux potentials from three continental sites in the U.S. *Chemosphere,* **38(9)** 2189-2204.

Hoechst AG (1980) *Bull. Environ. Contam. Toxicol.*, **24**, 684-691.

Hoechst AG (1988a) Unveroeffentlichte Untersuchung (V 88.0514). Unpublished report.

Hoechst AG (1988b) Unveroeffentlichte Untersuchung (V 88.0254). Unpublished report.

Hoechst AG (1990) Sicherheitsdatenblatt Camphen (06.07.1990) Unpublished report.

Hoechst AG (1991a) Product information Camphen der Abt. Marketing Chemikalien. Unpublished Report.

Hoechst AG (1991b) Unveroeffentlichte Untersuchung (S 91/484) Unpublished report.

Hoechst AG (1991c) Unveroeffentlichte Untersuchung, Analytisches Laboratorium. (Nr. 229-91(B)) Unpublished report.

Hoechst AG (1991d) Unveröffentl. Unters. im Auftrag der Hoechst AG (Ber.-Nr. 91.1203) Unpublished report.

Hoechst AG (1991e) Unveröffentl. Unters. (Ber.-Nr. 91.0246). Unpublished report.

Hoechst AG (1991f) Unveröffentl. Unters. (Ber.-Nr. 91.0475). Unpublished report.

Hoechst AG (1992) LPT Laboratory of Pharmacology and Toxicology, Report No. 7263/92 (HOE 92.1167) LPT Research. Report No. 7114/91.

Hoechst AG (1993) Dr. Noack (92.1127). Unpublished report.

Hughes P. R. (1975) Pheromones of *Dendroctonus*: origin of *alpha*-pinene oxidation products present in emergent adults. *Journal of Insect Physiology*, **21**, 687.

Ishida T., Asakawa Y., Takemoto, T. and T. Aratani (1981) Terpenoids biotransformation in mammals III: Biotransformation of *alpha*-Pinene, *beta*-Pinene, Pinane, 3-carene, carane, myracene, and p-cymene in rabbits. *Journal of Pharmaceutical Sciences*, **70(40),** 406-415.

Jagannath D.R. (1984) Mutagenicity evaluation of *alpha*-pinene. Private communication. Unpublished report.

Jones L J. (2003) Nootkatone, Verbenone: 28-Day repeated dose single dose toxicity study in the rat. Safepharm Project No. 1834/003. Private Communication to FEMA.

Kawai T., Yasugi T., and K. Mizunuma (1992) The comparative evaluation of urinanalysis and blood analysis as means of detecting exposure to organic solvents at low concentration. *Archives of Occupational and Environmental Health,* **64**, 223-234.

Kay J. H. (1963) Subacute vapor inhalation toxicity of turpentine. Industrial Bio-Test Laboratories, Inc., Unpublished report.

Klimisch H. J., Andreae, M., and U. Tillman (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Journal of Regulatory Toxicology and Pharmacology*, **25**, 1-5.

Kodama R., Okubo, A., Sato, E., Araki, K., Noda, K., Ide, H. and Ikeda (1977) Pinene concentrations in forest canopies. *Oyo Yakuri,* **13**, 885.

Kohn F. (1962) Acute inhalation toxicity study on turpentine. Industrial Bio-Test Laboratories, Inc., Unpublished report.

Koppel C., Tenczer, J., Tonnesmann, U., Schirop, T. and K. Ibe (1981) Acute poisoning with pine oil-metabolism of monoterpenes. *Archives of Toxicology*, **49**, 73-78.

Koistinen J., Lehtonen, M., Tukia, K., Soimasuo, M., Lahtipera, M. and A. Oikari (1998) Identification of liphophilic pollutants discharged from a Finnish pulp and paper mill. *Chemosphere*, **37(2)**, 219-235.

Krause C., Mailahan W., Nagel R., Seifert B. and D. Ullrich (1987) Occurrence of volatile organic compounds in the air of 500 homes in the Federal Republic of Germany. *Indoor Air,* **1**, 102-106.

Lehman-McKeeman L.D. and D. Caudill (1994) d-Limonene induced hyaline droplet nephropathy in *alpha*-2-microglobulin transgenic mice. *Fundam. Appl. Toxicol*., **23**, 562-568.

Levin J.O., Eriksson K., Falk A. and A. Lof (1992) Renal elimination of verbenols in man following experimental a-pinene inhalation exposure. *International Archives of Occupational and Environmental Health*, **63**, 571-573.

Li J. and Perdue, E.M. (1995) Syracuse Research Corporation.

Long K W. (2000) Gum turpentine: Toxicity to the green alga Selenastrum capricornutum. Report No. BL/7031/B. Unpublished Report.

Long K. W. (2001a) Gum turpentine: Acute toxicity to Daphnia magna. BL7032/B. Unpublished Report.

Long K. W. (2001b) Gum turpentine: Determination of 28-day ready biodegradability (Closed Bottle Sturm Test). Report No. Bl7034/B. Unpublished report.

Long K W.J. (2000) Gum turpentine: Toxicity to the green alga Selenastrum capricornutum. Report No. BL/7031/B. Unpublished Report.

Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. Environmental Toxicology and Chemistry, 15(9), 1627-1637.

Madsen T (1993a) Biodegradation of *alpha*-pinene. Project 303068, Water Quality Institute, Horsholm, Denmark.

Madsen T (1993b) Biodegradation of 3-carene. Project 303068, Water Quality Institute, Horsholm, Denmark.

McGowan and Mellors (1986) Bull. Environ. Contam. Toxicol., **36(6),** 881-887 and Heitmueller, Hollister, and Parrish (1981) Bull. Environ. Contam. Toxicol., **27(5),** 596-604.

McKibben R. (1979) A History of SCM Organic Chemicals. Millennium Chemicals. Unpublished report.

Merck Index (1996) 12th edition, Susan Budavari, editor, Merck & Co. Inc. Whitehouse Station, NJ.

Meylan W. (1993-1995a) LOGKOW, Syracuse Research Corporation.

Meylan W. (1993-1995b) WSKOW, Syracuse Research Corporation.

Meylan W. (1994a) BIOWIN, Syracuse Research Corporation.

Meylan W. (1994-1995b) AOPWIN, Syracuse Research Corporation.

Meylan W. (1994-1995c) MPBP, Syracuse Research Corporation.

Misra G., Pavlostathis, S. G., Perdue, E. M., and Araujo, R. (1996) Aerobic biodegradation of selected monoterpenes. *Applied Microbiology and Biotechnology,* **45**, 831-838.

Moreno O.M. (1972a) Acute oral toxicity in rats. Unpublished report to RIFM.

Moreno O. M. (1972b) Acute dermal toxicity of alpha-pinene in rabbits. Unpublished report to RIFM.

Moreno O. M. (1972c) Acute dermal toxicity of turpentine oil in rabbits. Unpublished report to RIFM.

Moreno O.M. (1974a) Acute oral toxicity in rats. Unpublished report to RIFM.

Moreno O. M. (1974b) Acute dermal toxicity of camphene in rabbits. Unpublished report to RIFM.

Moreno O.M. (1975a) Acute oral toxicity in rats Unpublished report to RIFM.

Moreno O. M (1975b) Acute dermal toxicity of *beta*-pinene in rabbits. Unpublished report to RIFM.

Morgareidge K. (1973a) Teratologic evaluation of FDA 71-28 in mice. Contract No. FDA 71-260. Unpulished Report.

Morgareidge K. (1973b) Teratologic evaluation of FDA 71-28 in hamsters. Contract No. FDA 71-260. Unpulished Report.

Morgareidge K. (1973c) Teratologic evaluation of FDA 71-28 in rats. Contract No. FDA 71-260. Unpulished Report.

Nabholz V. and G. Cash (1998) ECOSAR, U.S. Environmental Protection Agency, OPPT Risk Assessment Division.

National Resources Institute (1995) Gum naval stores: turpentine and rosin from pine resin. In Non-wood forest products 2, publisher: Food and Agriculture Organization of the United Nations, Rome.

National Toxicology Program (NTP Draft) (2006) Initial study results from a 90 day toxicity study on alpha-pinene in mice and rats. Study number SRL-13 wk 03AP/M and SRL-13 wk 03AP/R

Occupational Health and Environmental Control (OHEC) (1974)Subpart G Sec. 1910.93. Air Contaminants *Federal Register,* 39(125), 235-240.

Opdyke D. (1978) Monographs on fragrance raw materials. *Food and Cosmetic Toxicology*, **16(IV)**, 853, 859.

Oser B.L. and Hall R.L. (1977) Criteria employed by the expert panel of FEMA for the GRAS evaluation of flavouring substances. *Food and Cosmetic Toxicology,* **15**, 457-466.

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).

Petersen G.I. (1993) Growth inhibition test of *alpha*-pinene with the micro algae Selenastrum capricornutum, Project 303068, Water Quality Institute, Horsholm, Denmark.

Piccirillo V.J. (1984) Fourteen day subacute toxicity study in the rat. Private communication.

Renwick J., Hughes, P., and I. Krull (1976) *Science*, **191**, 199.

Rinne J., Hakola, H., Laurila, T. and Ü. Rannik (2000) Canopy scale monoterpene emissions of *Pinus sylvestris* dominated forests. *Atmos. Environ.*, **34**, 1099-1107.

Rockwell P. and I. Raw (1979) A mutagenic screening of various herbs, spices, and food additives. *Nutrition and Cancer*, **1(4),** 10-15.

Roy A. K. and O.W. Neuhaus (1967) Androgenic control of a sex dependent protein in rat. *Nature,* **214**,618-620.

Rudio J. (1999a) Inherent biodegradability of pinene *alpha* according to OECD Guideline No. 302C.Private communication to FFHPVC.

Rudio J. (1999b) Ready biodegradability of pinene *alpha* according to OECD Guideline No. 301F. Private communication to FFHPVC.

Samfield M. M. (1992) Indoor air quality database for organic compounds, EPA-600/13.

Sasaki Y.F., Imanishi, H., Ohta, T. and Shirasu, Y. (1989) Modifying effects of components of plant essence on the induction of sister-chromatid exchanges in cultured Chinese hamster ovary cells. *Mutation Research*, **226**, 103-110.

Sittig M. (1977) How to remove pollutants and toxic materials from air and water. Noyes Data Corporation. Unpublished report.

Sjödin K., Persson, M., Fäldt, J., Ekberg, I. and A.-K. Borg-Karlson (2000) Occurrence and correlations of monoterpene hydrocarbon enantiomers in *Pinus sylvestris* and *Picea abies*, *Journal of Chem. Ecol*., **26(7)** 1701-1720.

Southwell I.A., Flynn T.M. and R. Degabriele (1980) Metabolism of *alpha*- and *beta*-pinene, p-cymene and 1,8-cineole in the brushtail possum, Trichosurus vulpecula. *Xenobiotica*, **10(1),** 17-23.

Sperling F., Marcus, W. and C. Collins (1967) Acute effects of turpentine vapor on rats and mice. *Toxicology and Applied Pharmacology,* **10**, 8-20.

Stofberg J. and F. Grundschober (1987) The consumption ratio and Food Predominance of flavoring materials. *Perfumer & Flavorist,* **12**, 27-56.

Stofberg J. and J. C. Kirschman (1985) The consumption ratio of flavoring materials: A mechanism for setting priorities for safety evaluation. *Food and Chemical Toxicology,* **23**, 857-860.

Strasser J., Jr., Charbonneau M., Borghoff S.J., Turner M.J. and J.A. Swenberg (1988) Renal protein droplet formation in male Fischer 344 rats after isophorone (IPH) treatment. *Toxicologist,* **8,** 136.

Swarbrick R.H. (2001) Gum turpentine: Acute toxicity to rainbow trout (Oncorhynchus mykiss). BL7033/B. Unpublished Report.

Swenberg J. A., Short B., Borghoff S., Strasser J. and M. Charbonneau (1989) The comparative pathobiology of nephropathy. *Toxicol. Appl. Pharmacol.,* **97,** 35-46.

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.

Troy W.R. (1977) Doctoral Dissertation: The comparative respiratory irritation potential of fourteen fragrance raw materials. Unpublished report to RIFM.

Untermann R.D., Lynch K.R., Nakhasi H.L., Dolan K.P., Hamilton J.W., Cohn D.V., and P. Feigelson (1981) Cloning and sequencing of several *alpha*-2-microglobulin cDNAs. *Proc. Nat. Acad. Sci.,* **78,** 3478-3482.

von Skramlik E. (1959) On the toxicity and compatibility of essential oils. *Die Pharmazie*, **14**, 435-445.

Wang K.S. and R.B. Hodgetts (1998) The analysis of regulatory sequences required for the high level induction of rat *alpha*-2-microglobulin gene expression by dexamethasone. *J. Molec. Endocrin*., **20(1),** 129-139.

Webb D.R., Kanerva, R.L., Hysell, D.K., Alden, C.L., and L.D. Lehman-McKeeman (1990) Assessment of the subchronic oral toxicity of *d*-limonene in dogs. *Food and Chemical Toxicology, **28***, 669-675.

White Jr. R.A. and M. Agosin (1980) Metabolism of *alpha*-pinene by rat liver reconstituted cytochrome P-450 systems. *Development in Biochemistry*, **13**, 85-88.

Zhu Y., Cheng X., Wang L., Wei X., Yang C., and Tong Z. (2003) Measurement and correlation of saturated vapor pressure of pinane. *Gaoxiao Huaxue Gongcheng Xuebao*, **17**, 564-568.

Zlatkis A., Lichtenstein H.A., Tishbee A., Bertsch W., Shunbo F. and H.M. Liebich (1973) Concentration and analysis of volatile urinary metabolites. *Journal of Chromatographic Science*, **11**, 299-302.