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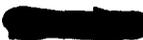
The Terpene Consortium

Test Plan For Bicyclic Terpene Hydrocarbons

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

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FFHPVC Terpene Consortium Registration Number 

Submitted to the EPA under the HPV Challenge Program by:
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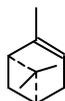
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The HPV Challenge Test Plan for Bicyclic Terpene Hydrocarbons

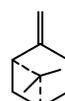
1 Identity of Substances



alpha-Pinene
C₁₀H₁₆

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
C₁₀H₁₆

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

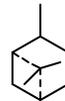
CAS No. 127-91-3



Camphene
C₁₀H₁₆

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

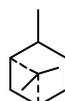
CAS No. 79-92-5



cis-Pinane
C₁₀H₁₈

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

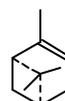
CAS No. 6876-13-7



Dihydropinene
C₁₀H₁₈

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

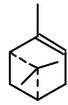
CAS No. 473-55-2



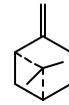
l-alpha-Pinene
C₁₀H₁₆

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



+



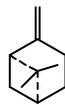
92-97%

1-7%

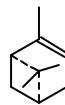
Terpenes and terpenoids, turpentine oil, *alpha*-pinene fraction

Synonyms: Oil of turpentine, *alpha*-pinene fraction

CAS No. 65996-96-5



+



+

other terpene hydrocarbons

78-81%

8-10%

Terpenes and terpenoids, turpentine oil, *beta*-pinene fraction

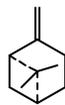
Synonyms: Oil of turpentine, *beta*-pinene fraction

CAS No. 65996-97-6



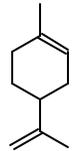
59%

+



24%

+



5%

other terpene hydrocarbons

Turpentine oil

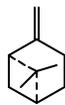
Synonyms: Oil of turpentine, Turpentine

CAS No. 8006-64-2



60-65%

+



25-35%

+

5-8% other monocyclic terpene hydrocarbons

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 C₁₀ 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. *alpha*-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]. *alpha*-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 consumed 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 C₁₀H₁₆ 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 C₁₀ 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/m³ [Koistinen *et al.*, 1998; Samfield, 1992; Krause *et al.*, 1987], 500-1200 ug/m³ [Kodama *et al.*, 1977], and 200,000-500,000 ug/ m³, 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/m³. 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/m³ *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 $1\{10 \text{ ug/m}^3 \times 3 \text{ m}^3/\text{hr} \times 24\text{hrs/day} \times 0.6 \text{ (absorption rate)} \times 10^{-3} \text{ ug/mg}\}$. In a rural environment in which atmospheric concentrations of *alpha*-pinene are routinely 500 ug/m³, 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 diastereoisomers 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 C₁₀ hydrocarbons that contain an exocyclic methylene function. A structurally related C₁₀ 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 $\mu\text{gChr}^{-1}\text{gdw}^{-1}$ (micrograms carbon per hour per gram dry weight) for *alpha*-pinene, 0.1 to 24 $\mu\text{gChr}^{-1}\text{gdw}^{-1}$ for *beta*-pinene and 0.2 to 39 $\mu\text{gChr}^{-1}\text{gdw}^{-1}$ 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 $\mu\text{gCm}^{-2}\text{hr}^{-1}$ (micrograms carbon per m^2 per hour), for *beta*-pinene, 9, 55 and 3 $\mu\text{gCm}^{-2}\text{hr}^{-1}$ and for camphene, 32, 130 and 12 $\mu\text{gCm}^{-2}\text{hr}^{-1}$. 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 ($82 \mu\text{gCm}^{-2}\text{hr}^{-1}$), *beta*-pinene ($22 \mu\text{gCm}^{-2}\text{hr}^{-1}$) and camphene ($58 \mu\text{gCm}^{-2}\text{hr}^{-1}$) 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 C₁₀ 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 C₁₀ 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/m³ 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/m³ and 450 mg/m³, respectively. Uptake of *delta*-3-carene was 61% and 70% at 10 mg/m³ and 450 mg/m³, respectively. There was no difference in pulmonary uptake between the enantiomers (+)- and (-)-*alpha*-pinene at 450 mg/m³. 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/m³) 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 atmosphere containing 6.4 or 3.2 ppm (24 mg/m³ and 12 mg/m³) of a mixture of volatile organic substances. At 6.4 ppm, the air

concentration of *alpha*-pinene was 0.139 ppm (0.775 mg/m³). 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, 0.070, 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

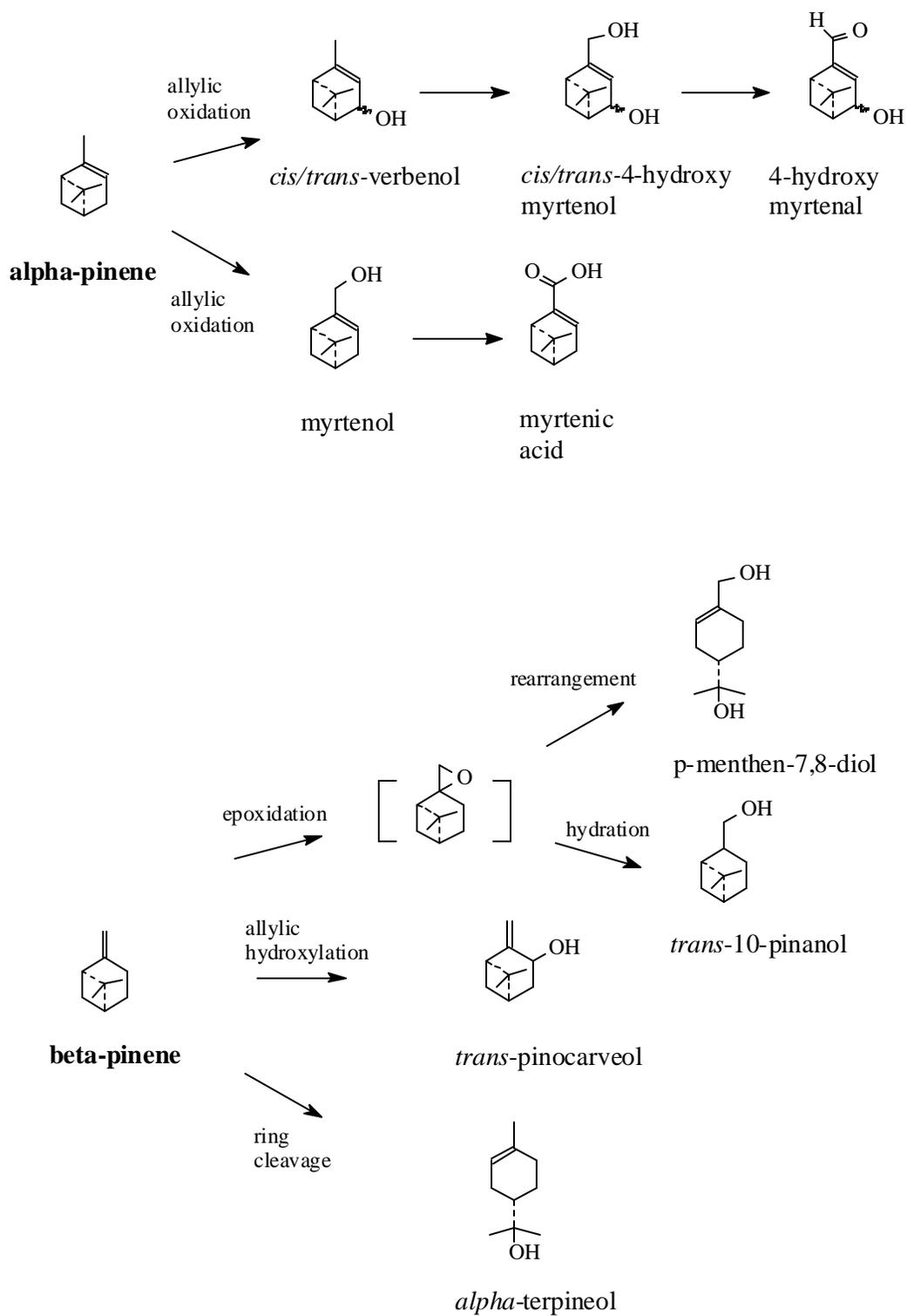
In humans, bicyclic terpene hydrocarbons are metabolized by cytochrome P-450 (CYP-450) induced C-oxidation to produce polar oxygenated metabolites that are conjugated and excreted primarily in the urine (see Figure 1). Analysis of urinary metabolites eliminated within 4 hours following a 2-hour exposure 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/m³ and 1.7% at 450 mg/m³. 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/m³ 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-hydroxymyrtanol, formed by methyl group hydroxylation of *cis*- and *trans*-verbenol. *trans*-4-Hydroxymyrtanol 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 myrtanol, 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, (\pm)-*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 housed 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 (\pm)} 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 C₂ 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 C₂ 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 *Dendroctonus* [Hughes, 1975] bark beetles metabolize *alpha*-pinene to verbenol and myrtenol with verbenol being further oxidized to verbenone in *Dendroctonus*.

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 pentoxoresorufin-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 C₁₀ 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 °C), camphene (51-2 °C), and *cis*-pinane (-53 °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 °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 °C). *1-alpha*-Pinene is one stereoisomer of *alpha*-pinene and therefore would have the same melting point (-55 °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 °C @ 760 mm), *beta*-pinene (165-166 °C @ 760 mm), camphene (158.5-159.5 °C @ 760 mm), *cis*-pinane (169 °C @ 760 mm) and dihydropinene (164.5-165 °C @ 760 mm) [Merck Index, 1996; CRC Handbook of Chemistry and Physics, 1986]. *1-alpha*-Pinene is one stereoisomer of *alpha*-pinene and therefore would have the same boiling point (155-156 °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 °C (0.18-0.41 mm Hg) for *alpha*- and *beta*-pinene and 3.8 kPa (2.8 mm Hg) for camphene at 20 °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 °C, respectively [Hoechst AG, 1991b]. The range of vapor pressure at ambient temperature is fairly narrow. However, vapor pressure measurement should be conducted on at least one representative member of the group, *alpha*-pinene, to confirm the model. In the event of close agreement with the calculated value, no further testing would be necessary.

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 °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 °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

Vapor pressure measurements to confirm the SAR model should be conducted on at least one representative member of the group, *alpha*-pinene, in accordance with OECD Guideline 104. In the event of close agreement with the calculated value, no further testing would be necessary.

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 NO₃ 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.

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 environment 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

None.

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 *1-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° 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 *1-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 *1-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

None.

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 measured in different animal species. The acute LC50 was reported to be 13,500 mg/m³ in rats, 13,500 mg/m³ in guinea pigs, and 9000 mg/m³ 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/m³ for 1 to 6 hour exposures and the LC50 for a 2-hour exposure in Swiss-Webster mice is 29,000 mg/m³ [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].

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*. No additional *in vitro* and *in vivo* assays are requested for this chemical category.

3.4.3 Repeat Dose Toxicity

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.

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].

The repeat-dose study for camphene provides a NOAEL of 250 mg/kg bw/day [Hoechst AG, 1991f]. It is expected that *alpha*- and *beta*-pinene, if subjected to a 28-day study at similar dose levels would exhibit *alpha*-2-microglobulin nephrotoxicity in male rats and would be expected to exhibit a NOAEL of at least 250 mg/kg bw/day.

3.4.4 Reproductive Toxicity

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 C₁₀H₁₆ 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 mesh-bottom cages in a temperature- and humidity-controlled room. They 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. When this data is combined with the fact that no adverse effects were observed to the reproductive organs in a 28-day 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 are not reproductive toxicants.

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

None.

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 <i>alpha</i> -Pinene	A	A	Test, Calc, R	A, Calc, R	A, Calc
CAS No. 127-91-3 <i>beta</i> -Pinene	A	A	Calc	Calc	A, Calc
CAS No. 79-92-5 Camphene	A	A	A, Calc	Calc	A, Calc
CAS No. 6876-13-7 <i>cis</i> -Pinane	A	A	Calc	Calc	Calc
CAS No. 473-55-2 Dihydropinene	A	A	Calc	Calc	Calc
CAS No. 7785-26-4 <i>l</i> - <i>alpha</i> -Pinene	A	A	Calc	Calc	Calc
CAS No. 65996-96-5 Terpenes & Terpenoids, Turpentine oil, <i>alpha</i> - Pinene fraction	R	R	Calc	Calc	Calc
CAS No. 65996-97-6 Terpenes & Terpenoids, Turpentine oil, <i>beta</i> - 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 <i>alpha</i> -Pinene	Calc	NA	A, Calc, R	Calc
CAS No. 127-91-3 <i>beta</i> -Pinene	Calc	NA	Calc	Calc
CAS No. 79-92-5 Camphene	Calc	NA	A, Calc	Calc
CAS No. 6876-13-7 <i>cis</i> -Pinane	Calc	NA	Calc	Calc
CAS No. 473-55-2 Dihydropinene	Calc	NA	Calc	Calc
CAS No. 7785-26-4 <i>l-alpha</i> -Pinene	Calc	NA	Calc	Calc
CAS No. 65996-96-5 Terpenes & Terpenoids, Turpentine oil, <i>alpha</i> - Pinene fraction	Calc	NA	Calc	Calc
CAS No. 65996-97-6 Terpenes & Terpenoids, Turpentine oil, <i>beta</i> - 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 <i>alpha</i> -Pinene	A, Calc	A, Calc	A, Calc
CAS No. 127-91-3 <i>beta</i> -Pinene	A, Calc	A, Calc	A, Calc
CAS No. 79-92-5 Camphene	A, Calc	A, Calc	A, Calc
CAS No. 6876-13-7 <i>cis</i> -Pinane	Calc	Calc	Calc
CAS No. 473-55-2 Dihydropinene	Calc	Calc	Calc
CAS No. 7785-26-4 <i>l-alpha</i> -Pinene	Calc	Calc	Calc
CAS No. 65996-96-5 Terpenes & Terpenoids, Turpentine oil, <i>alpha</i> -Pinene fraction	Calc	Calc	Calc
CAS No. 65996-97-6 Terpenes & Terpenoids, Turpentine oil, <i>beta</i> -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 <i>In Vitro</i>	Genetic Toxicity <i>In Vivo</i>	Repeat Dose Toxicity	Reproductive Toxicity	Developmental Toxicity
CAS No. 80-56-8 <i>alpha</i> -Pinene	A	A	R	R	A	A
CAS No. 127-91-3 <i>beta</i> -Pinene	A	A	R	A	A	A
CAS No. 79-92-5 Camphene	A	A	A	A	A	A
CAS No. 6876-13-7 <i>cis</i> -Pinane	R	R	R	R	R	R
CAS No. 473-55-2 Dihydropinene	R	R	R	R	R	R
CAS No. 7785-26-4 <i>l</i> - <i>alpha</i> -Pinene	R	R	R	R	R	R
CAS No. 65996-96-5 Terpenes & Terpenoids, Turpentine oil, <i>alpha</i> -Pinene fraction	R	R	R	R	R	R
CAS No. 65996-97-6 Terpenes & Terpenoids, Turpentine oil, <i>beta</i> -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

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