Turpentine
(Turpentine Oil, Wood Turpentine, Sulfate Turpentine, Sulfite Turpentine)
[8006-64-2]

Review of Toxicological Literature

February 2002

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Executive Summary

Nomination

Turpentine was nominated by the International Union, United Auto Workers for chronic toxicology and carcinogenesis studies based on widespread human exposure and reports of kidney toxicity in chronically exposed humans. Furthermore, the nominated studies would potentially offer mechanistic insight into the human relevance of chemically induced $\alpha 2\mu$-globulin formation and resulting kidney toxic and carcinogenic responses observed in exposed animals.

Non-toxicological Information

Two CAS RNs were identified for turpentine: [9005-90-7] refers to the oleoresin obtained from trees of the species *Pinus*; [8006-64-2] refers to essential oils (turpentine oil) derived from the oleoresin through the distillation process. Turpentine oil is the focus of this report.

Turpentine is characterized by both its starting material and production process. All turpentines are produced from *Pinus* spp. trees. Turpentine oil is derived from the oleoresin (balsam) collected from the tree. Distillation of this material produces turpentine oil and the solid rosin. Steam-distilled (wood) turpentine is derived from finely chopped wood chips and processed by either steam distillation or solvent extraction. Sulfate and sulfite turpentines are by products of the kraft and sulfite pulping processes, respectively. Destructively distilled wood turpentine is made by dry distillation of pinewood (primarily pine stumps), followed by purification.

Turpentine is a mixture of constituents. The type and amount of specific constituents is dependent on the type of pine tree, the geographical location of the trees, and the season of tree harvest. Turpentine produced in the United States is made up primarily of α-pinene (75 to 85%) with varying amounts of β-pinene (up to 3%), camphene (4 to 15%), limonene (dipentene, 5 to 15%), 3-carene, and terpinolene (percentages not provided).

The production of turpentine oil in the United States peaked in the 1950s. Georgia is the only remaining state that continues to collect pine balsam for the production of turpentine oil. Steam-distilled wood turpentine was produced in the United States in the 1960s and 1970s. Since that time most of the turpentine produced in the United States is the by-product of the kraft pulping process, sulfate turpentine. In 1999, total domestic production of turpentine was 20.7 million gallons. The production of destructively distilled wood turpentine is no longer of commercial importance. No additional information was available for sulfite turpentine. According to the U.S. EPA (2000), aggregate production of turpentine oil ranged from between 100 and 500 million pound in the United States for the 1998 reporting cycle.

Turpentine was formerly the most widely used paint and varnish thinner and is still used in some paints and coatings. The use of less expensive petroleum-based products has replaced the use of turpentine in paints. When turpentine is used today, it is most likely in specialty applications such as spray painting, pottery and ceramic coatings, artist's paints, and naval paints. Turpentine is sometimes present in shoe and furniture polishes, and is used as a metal cleaner. Currently, the major use of turpentine is as a raw material for the chemical industry. Terpenes and other compounds extracted from turpentine can be used for such products as tires, plastics, adhesives, flavors and fragrances, cosmetics, paints, and pharmaceuticals. The value of turpentine represents about 25% of the value of all aroma chemicals produced for both sale and for internal use each year. Past uses for turpentine include printing processes, hairdressing formulations, and as medicinals.

Human Data

Occupational exposure to turpentine is expected to occur as a result of pulp and paper processes, turpentine production, the production of other chemicals from turpentine, use as a flavoring or fragrance agent, use in coatings, use as a metal cleaner, or as a solvent. The general public may be

exposed to turpentine through foods, personal care products, household products, and external and internal medications. Terpenes, volatile components of turpentine, are emitted into the atmosphere by trees. Low-level environmental exposure to these terpenes would occur in the proximity of pine forests.

The mean oral lethal dose of turpentine for humans ranges from 15 to 150 mL (~13 – 129 g; 95 – 947 mmol). Systemic toxicity to turpentine usually results in gastrointestinal (GI) irritation and central nervous system (CNS) depression within two to three hours of exposure; symptoms generally subside within 12 hours except in severe exposures. Acute exposure to high levels of turpentine for several hours results in ocular irritation, headache, dizziness, nausea, and tachycardia. Severe exposures may cause death. The National Institute for Occupational Safety and Health has calculated that a concentration of 800 ppm (4457 mg/m^3) is immediately dangerous to life and health.

Dermal Sensitization
Occupational allergic dermatitis is well documented and is often related to a change in the source of turpentine. Different methods of turpentine production, source of trees, and season of harvest will yield formulations with different chemical compositions and possibly different toxicological effects.

Changes in the patterns of reactivity to turpentine over the last half century were reported. In the 1970's, there was an apparent decline in the reactivity to turpentine. The decline in reactivity presumably was due to the replacement of turpentine with water-soluble paints and less expensive paint thinners. In 1996, positive reaction rates to turpentine more than tripled over the rates of the previous year (from 0.5% in 1995 to 1.7% in 1996). The rate increased the following year to 3.1%. The high rate of reactors may be due to increased use of turpentine in liniments and creams, possible cross reactivity with other allergens such as ragweed, chrysanthemums, peppermint, and bergamot oil, or possibly linked to increased use of tea tree oil.

Chronic Effects
Chronic effects associated with occupational exposures to turpentine include cerebral atrophy, behavioral changes, anemia and bone marrow damage, glomerulonephritis, and dermatitis. Urinary disturbances, albuminuria, and urinary casts were observed in workers exposed to paints and varnishes. However, renal damage associated with occupational exposures to turpentine was transient and reversible.

A number of epidemiology studies have been completed that were associated with the pulp and paper industry. Cancers considered included lung, lymphoproliferative diseases (Hodgkin's disease, multiple myeloma, leukemia, lymphosarcomas), and cancers of the digestive organs. These studies were confounded by other possible chemical exposures. Without job-exposure matrices, it is difficult to pinpoint exposures to specific chemicals and corresponding risks of developing cancers.

In a more recent investigation of the potential association of parental exposures to various chemicals and the occurrence of neuroblastoma in their children, an association between paternal turpentine exposure and elevated incidence rates was found for neuroblastoma (odds ratio = 10.4; 95% confidence interval = 2.4 to 44.8).

Animal Studies
Chemical Disposition, Metabolism, and Toxicokinetics
Turpentine is readily absorbed through the GI and respiratory tracts, and skin. Turpentine, as a lipophilic substance, accumulates in fatty tissues. The highest concentrations of turpentine following inhalation by rats were found in the spleen, kidneys, brain, and peripheral and perinephric fat. Liver microsomal epoxide hydrase and uridine diphosphoglucuronosyl transferase activities were elevated during chronic turpentine exposures. While some turpentine may be eliminated unchanged through

the lungs, most turpentine and its metabolites are eliminated through the urinary tract as glucuronides.

Acute Toxicity
Signs of acute inhalation toxicity for turpentine include salivation, weakness, incoordination, bloody nasal discharge, paraplegia, ataxia, tremor, convulsions, tachypnea, decreased tidal volume, coma, and death due to sudden apnea. High-level exposures cause irritation of the skin, nose, and mucosal membranes. Central nervous system depression is accompanied by an increased respiration rate and decreased tidal volume. Systemic effects include damage to the kidney and liver. Hyperplasia was demonstrated within 48 hours of a single cheek painting in the hamster cheek pouch model. Thirty percent turpentine in acetone elicits a moderate degree of skin irritation free from ulcer formation.

Short-Term and Subchronic Toxicity
Inhalation of turpentine (715 ppm; 3985 mg/m^3), four hours per day, 45 or 58 days failed to result in any hematological changes in guinea pigs, though slight changes in the liver and moderate scattered tubular degeneration in the kidneys were observed. Subchronic inhalation of turpentine (300 ppm; 1670 mg/m^3) in rats resulted in the accumulation of α-pinene in brain and perinephric fat. At higher concentrations (estimated at 5000 to 10,000 mg/m^3 or 900 to 1800 ppm) for up to 293 hours over a period of time ranging to 14 months provided no histological evidence of nephritis or chronic Bright's disease. There were foci of pneumonitis and extensive lung abscesses in many of these animals.

Turpentine injections (intramuscular for rats; subcutaneous for mice) resulted in anemia in both species. Mice were injected subcutaneously into the intrascapular fat pad once per week for six weeks. Rats developed abscesses at the site of injections. Blood volumes were reduced relative to body weight. Intradermal injections once per week for three weeks did not result in adverse pathological liver findings in male Sprague-Dawley rats.

Hyperplasia was noted in almost all studies involving dermal application of turpentine. In one study, inflammation, measured by ear swelling, was found to be similar between rats injected with turpentine or with 12-O-tetradecanoylphorbol-13-acetate. Hypoferremia was observed in studies where inflammation was induced by turpentine.

In situ exposures produced perinephritis, acute tubular or cortical necrosis and unilateral or bilateral glomerular fibrinogen deposition. Depositions of immunoglobulin G and β1C were found on glomerular basement membranes and mesangium of both the treated and contralateral kidney.

Chronic Toxicity
No chronic toxicity studies of turpentine were identified.

Synergistic/Antagonistic Effects
Turpentine, in combination with CCl_4, resulted in increased fibrosis and less steatosis than with CCl_4 alone. Turpentine alone failed to produce overt liver pathology. Turpentine treatment reduced parathion toxicity and carrageenan-induced edema in rats. Guinea pigs were protected from hypersensitivity reactions to 6-mercaptopurine.

Reproductive and Teratological Effects
In a single teratology study exposing pregnant rats to turpentine vapors for ten minutes, twice a day, on gestation days 17 through 21, exposed pups exhibited dyspnea and severe CNS depression at birth, but no gross histopathological abnormalities in the brain.

Carcinogenicity
No carcinogenicity studies of turpentine were identified.

Initiation/Promotion Studies
Turpentine demonstrated mild promotional characteristics, relative to croton oil, when applied to the backs or ears previously initiated by 7,12-dimethylbenz(a)anthracene in mice. α-Pinene was reported to have similar promotional capabilities. In rabbits, dermal application of turpentine as a hyperplasia-inducing agent, enhanced cottontail rabbit papilloma virus-induced development of papilloma. The promoting effect of turpentine was not observed after initiation of mouse ears by ultraviolet radiation.

Anticarcinogenicity
Turpentine failed to act as an anticarcinogen in either of the two studies identified.

Immunotoxicity
Electron microscopic studies of macrophages of granulomas in rats intramuscularly exposed to turpentine revealed many inclusions, including lamellar bodies. It was concluded that quantitative and qualitative differences exist between turpentine-induced inflammation and other models of inflammation.

Other Data
Turpentine treatment, either by injection (intramuscularly, intradermally, and subcutaneously) or dermally, resulted in an inflammatory reaction that, under certain conditions, progresses to hyperplasia. Several acute-phase proteins were induced, such as sialyl-, galactosyl-, *N*-acetylglucosaminyltransferase, serum haptoglobin, and ceruloplasmin.

Turpentine was used in a number of animal model systems to distinguish non-malignant hyperplastic processes from malignant processes. Comparison of such characteristics as epidermal cell number, variations in epidermal thickness, presence of cellular inflammatory processes, or hyperplasia between turpentine or *d*-limonene treatments and neoplastic agents were made. Turpentine-induced hyperplasia models were also used to evaluate the discriminative ability of toluidine blue or immunohistochemistry examination for cytokeratin expression. In the former case, the authors were unable to induce a hyperplastic state. In the cytokeratin study, the neoplastic agent DMBA resulted in changes in both the high and low molecular weight cytokeratins. Cytokeratin levels were not affected by turpentine oil treatment.

No studies were identified related to the genotoxicity, cogenotoxicity, or antigenotoxicity of turpentine.

Structure-Activity Relationships
The acute toxicity of turpentine is similar to the acute toxicity of its constituents. It is thought that the *d*-enantiomers of the monoterpenes are more toxic than the *l*-enantiomers.

α- and β-Pinene
Most of the studies identified relative to α- and β-pinene were associated with human responses to one or several monoterpenes. No chronic or carcinogenicity studies were identified for α- or β-pinene. Both pinenes were listed as irritants to the skin, eye, and mucus membrane. α- And β-pinene are readily absorbed through the pulmonary system, skin, and intestines. Acute toxic effects are stated as similar to those resulting from turpentine exposures. The mean lethal dose of α-pinene was equivalent to four to six oz of turpentine (120 to 180 mL) (as cited); the probable oral human lethal dose of β-pinene ranged from 0.5 to 5 g/kg body weight.

Male rats treated with β-pinene demonstrated increased heptachlor mortality and benzo[a]pyrene hydroxylation. *In vitro*, α-pinene inhibited growth of viral Ha-*ras*-transformed rat liver epithelial cells (WB-*ras* cells); *in vivo*, α-pinene failed to demonstrate significant chemoprotective action in dimethylbenzanthracene-induced rat mammary carcinogenesis. α- And β-pinene have been investigated for their ability to enhance dermal penetration of several pharmaceuticals.

3-Carene
3-Carene, a minor component of turpentine, occurs in combination with α- and β-pinene in fumes produced in sawmills or from fumes from colophony solder. Pulmonary uptake of 3-carene increased linearly with exposure, approaching 70% for 225 and 450 mg/m^3 exposure levels during controlled two-hour exposures. 3-Carene persists in the blood with an extended half-life, suggesting a high affinity for adipose tissues. In a cross-sectional study of 38 workers from four joinery shops, it was concluded that exposures to sawmill fumes resulted in chronic lung function impairment.

d-Limonene
Limonene is considered a mild local irritant for eyes and the gastrointestinal tract and a skin sensitizer. It may be absorbed by all routes, though one report suggested that monoterpenes are poorly resorbed (as cited) from the gastrointestinal tract. Maximum blood levels of limonene were reached after ten minutes of dermal exposure in a foam bath. Inhalation studies demonstrated a relative pulmonary uptake in the range of 70%. Elimination is primarily through the kidneys (75 to 95%).

Limonene was tested in phase I clinical trials for the treatment of tumors. A partial response (11 months) was observed in one of ten breast cancer patients; three colorectal carcinoma patients progressed to prolonged stable disease. *d*-Limonene was well tolerated in cancer patients at doses that may have clinical activity. In a single case-control study, the consumption of citrus peel, a major source of limonene, was associated with a reduction in risk for squamous cell carcinoma in an older population located in the Southwestern United States.

Oral exposures to *d*-limonene produced a sex- (M) and species- (rat) specific nephropathy associated with the presence of hyaline droplet accumulation. Renal alterations included cytoplasmic basophilia of proximal convoluted tubule cells, tubular hyperplasia or atrophy, fibrosis of Bowman's capsule, and interstitial fibrolymphocytic response. Negative results were obtained in female rats and both sexes in mice. When dogs were dose with *d*-limonene close to the ED_{50} for emesis for six months, increases in relative (female) and absolute (male and female) kidney weights were observed without any other evidence of renal changes. *d*-Limonene exposures resulted in depressed cholesterol biosynthesis (rats and dogs), increased aminopyrine demethylase and aniline hydroxylase activity (rats), and reduced blood sugar levels (dogs). Alterations in the fatty acids of liver phospholipids were also observed in rats. At doses producing maternal toxicity (2363 mg/kg), an increase in abnormal bone formation (fetuses) and decrease in body weight gain (male offspring) was observed.

d-Limonene was tested by the NTP for general and genetic toxicity in male and female rats (F344/N) and mice ($B6C3F_1$). Although there was no evidence of carcinogenic activity of *d*-limonene in female F344/N rats or male and female B6C3F1 mice, there was clear evidence of carcinogenicity in male F344/N rats, as shown by the increased incidence of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney. Although there was sufficient evidence for carcinogenicity in experimental animals, the International Agency for Research on Cancer (IARC) concluded that there is inadequate evidence for the carcinogenicity of *d*-limonene in humans as *d*-limonene produces renal tubular tumors in male rats by non-DNA reactive α_{2u}-globulin-associated responses that were not relevant to humans.

Limonene has been tested for anticarcinogenic properties in a wide variety of chemically induced cancers such as dimethylbenzanthracene- and *N*-nitroso-*N*-methylurea-induced mammary tumors (rats), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone- and *N*-nitrosodiethylamine-induced gastric and lung neoplasias (rats and mice), *N*-nitrosobis(2-oxopropyl)amine-induced pancreatic cancer (Syrian golden hamster), and benzo[a]pyrene-induced skin cancer (mice). *d*-Limonene has also been effective in increasing the time to first tumor (latency), reducing the number of tumors per animal, and promoting regression of tumors already formed.

Research to date suggests that limonene is effective either prior to initiation or during promotion/progression stages of carcinogenesis. Effectiveness during the initiation phase is most likely due to modulations of phase I and phase II enzyme activities. Limonene in the diet (5%) resulted in a doubling in the hepatic glutathione-S-transferase activity. Both 3-methyl cholanthrene- and phenobarbital-inducible uridine diphosphoglucuronosyl transferase isozymes activities were also increased. Overall, increased cytochrome P450 enzymes, especially the CYP2B and CYP2C families, and epoxide hydrolase activities, were associated with exposures of rodents to *d*-limonene. Inhibitory effects of *d*-limonene during the promotion/progression phase were linked with an inhibition of post-translational isoprenylation of growth-controlling small G proteins such as p21*ras*.

d-Limonene exposures failed to result in observable mutations either *in vitro* or *in vivo*.

Oxidation products of *d*-limonene, (R)-(-)-carvone, (+)-limonene oxide, along with air oxidized *d*-limonene, were found to be potent sensitizers in the Freund complete adjuvant test and in the guinea pig maximization test. Treatment of BALB/c mice with *d*-limonene for nine weeks suppressed Concavalin-A, phytohemagglutinin, and lipopolysaccharide responses at week eight. Responses to keyhole limpet hemocyanin (KLH) were dependent on the order of challenge: *d*-limonene exposure prior to KLH resulted in a significant increase in antibody response; *d*-limonene after KLH produced a suppression of both primary and secondary anti-KLH responses. The results suggested that *d*-limonene has polyclonal activation action.

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1.0 Basis For Nomination

Turpentine was nominated by the International Union, United Auto Workers for chronic toxicology and carcinogenesis studies based on widespread human exposure and reports of kidney toxicity in chronically exposed humans. Furthermore, the nominated studies would potentially offer mechanistic insight into the human relevance of chemically induced $\alpha_{2\mu}$-globulin formation and resulting kidney toxic and carcinogenic responses observed in exposed animals.

2.0 Introduction

Turpentine
[CAS RN 8006-64-2 or 9005-90-7]

Turpentine is a name applied to numerous semi-fluid oleoresins obtained from coniferous trees. The substance obtained from these trees consists of 75 to 90 percent resin and 10 to 25 percent oil. When distilled, it yields turpentine ($C_{10}H_6$). Turpentine is a mixture of terpenes and essential oils, which vary in percentage based on geographic location, tree species, and the distillation process.

The nomenclature for turpentine is both confusing and inconsistent. Two CAS RNs currently in use for turpentine have been located: 8006-64-2 and 9005-90-7. CAS RN 8006-64-2 has the greatest quantity of information regarding turpentine. The synonyms used are consistent with the Naval Stores Act of 1923 (gum spirits, gum turpentine, spirits of turpentine, oil of turpentine). The definition provided for CAS RN 9005-90-7 is: "Oleoresin from *Pinus* species particularly *Pinus palustris*, Pinaceae." Synonyms for this citation include gum turpentine, pine gum, and pine resin. While the term turpentine gum has been used to refer to the essential oils distilled from the oleoresin of the pine tree (Coppen and Hone, 1995; Medicinal HerbFAQs, 2001), it has also been used to refer to the oleoresin itself (Mills, 2001). The Amended Mississippi Code of 1972 refers to crude turpentine gum as the oleoresin that is further processed to gum-spirit-of-turpentine and gum rosin (Mississippi Code, 1972).

From the context of most of the literature considered, reference to "gum" signifies the collection of oleoresin (or resins) from pine trees. Distillation of the oleoresin results in the volatile turpentine oil and the gum rosin. Rosin is the brittle, transparent, glossy, faintly aromatic solid that remains once all the turpentine oil has been extracted. If only a portion of the turpentine oil is removed, the remaining viscous mass is referred to as crude turpentine. Turpentine oil refers to the distillation product of the oleoresin only. CAS RN 9005-90-7 is specific for the oleoresin. There is little toxicology information associated with the oleoresin and therefore it will not be covered in this report.

Turpentine is often classified by its means of production (i.e., steam-distilled, destructively-distilled, sulfate-distilled, or sulfite-distilled turpentine). There is considerable difference between the types of turpentine in production levels and current use patterns, and thus in the

potential for human exposure. In evaluating the toxicological literature on turpentine, it was evident that many sources do not distinguish between the different types of turpentine.

2.1 Chemical Identification and Analysis[1]

Turpentine Oil [CAS RN 8006-64-2; mainly a mixture of α- and β-pinene with the formula $C_{10}H_{16}$; mol. wt. = 136.23] is also called:

Turpentine, oil (CA Index Name)	Oils, turpentine
Essential oils, turpentine	Spirits of turpentine
G 4134	Steam-distilled turpentine
Gum turpentine, oils	Sulfate turpentine
Gum spirits of turpentine	Turpentine oil, rectified
Oil of turpentine	Turpentine spirits
Oil of turpentine, rectified	Turpentine, steam distilled
Oils, essential, turpentine	Wood turpentine

Gum Turpentine [CAS RN 9005-90-7; mainly a mixture of abietic- and pimaric-type acids with smaller amounts of neutral compounds (Coppen and Hone, 1995)] is also called:

Turpentine (CA Index Name)	Pine gum
Galipot	Pine resin
Gum thus	Resins, pine
Gum turpentine	Resins, turpentine
Naval stores, turpentine	Skipidar
Petropine	Turpentine gum

Specifications for turpentine in the United States are described in American Society for Testing Materials (ASTM) D13-97 (ASTM, 2001a). These specifications include gum turpentine, steam-distilled wood turpentine, sulfate wood turpentine, and destructively distilled wood turpentine, as defined by the Naval Stores Act of 1923 (Stonecipher, 1955; cited by Santodonato, 1985):

> Gum Spirits of Turpentine: shall refer to the kind of spirits of turpentine obtained by distillation of oleoresin (gum) from living trees, and commonly known prior to the passage of the act as gum spirits, gum turpentine, spirits of turpentine, or oil of turpentine.
>
> Steam-Distilled Wood Turpentine: shall refer to the kind of spirits of turpentine obtained by steam distillation from the oleoresinous component of wood, whether

[1] Chemical information taken from Chemical Abstracts Registry File.

in the presence of the wood or after extraction from the wood and commonly known prior to the passage of the act as wood turpentine, steam-distilled turpentine, steam distilled wood turpentine or S.D. wood turpentine.

Destructively Distilled Turpentine: the kind of spirits of turpentine prepared from the distillate obtained in the destructive distillation (carbonization) of wood, commonly known prior to the passage of the act as destructively distilled wood turpentine or D.D. wood turpentine.

Sulfate Wood Turpentine: the kind of spirits of turpentine prepared from condensates that are recovered in the sulfate process of cooking wood pulp and commonly known as sulfate turpentine or sulfate wood turpentine.

These specifications are no longer widely used as they specify physical characteristics only and not chemical composition.

The amount of α-pinene, β-pinene, limonene, terpene alcohols, and other related terpene compounds present in turpentine can be determined using capillary gas chromatography, according to ASTM Method D6387-99 (ASTM, 2001b). Savolainen and Pfäffli (1978) specified the use of a gas chromatograph equipped with a glass column coated with Carbowax 20M for this purpose; nitrogen is used as the carrier gas.

Turpentine oil prepared by steam distillation (see **Section 3.0, Production Processes**) can be identified by the trace amounts of benzaldehyde it contains (Snider, 1945; cited by Gscheidmeier and Fleig, 1996).

2.2 Physical-Chemical Properties

Property	Information	Reference(s)
CAS RN 8006-64-2 (Turpentine):		
Physical State:	Colorless liquid	Budavari (1996)
Odor	Characteristic odor and taste	Budavari (1996)
Boiling Point (°C)	154-170	Budavari (1996)
Melting Point (°C)	-60 to -50	ChemFinder.com
Density (g/cm^3 at 20°C)	0.854-0.868	Budavari (1996)
Water Solubility	Insoluble	Budavari (1996)
Solubility in other solvents	Benzene, chloroform, ether, carbon disulfide, petroleum ether and oils	Budavari (1996)
CAS RN 9005-90-7 (Turpentine; rosin):		
Physical State	Yellowish, opaque, sticky mass	Budavari (1996)
Odor	Characteristic	ACGIH (1991)
Softening point (°C)	70-80	Coppen and Hone (1995)
Acid Number	160-170	Coppen and Hone (1995)
Water Solubility	Insoluble	Budavari (1996)

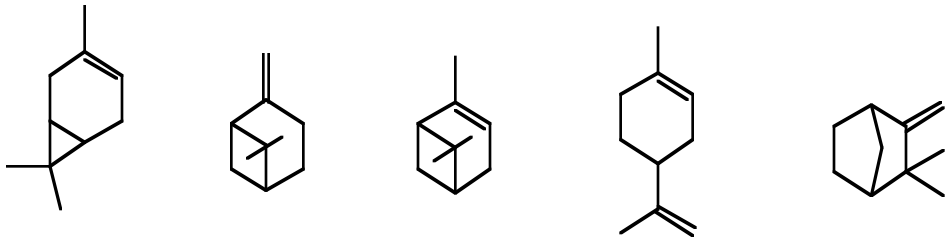
In turpentine produced in the United States, the major constituents are the volatile terpene hydrocarbons -- α-pinene (75 – 85%), β-pinene (up to 3%), camphene (4 - 15%), limonene (dipentene, 5-15%), 3-carene, and terpinolene (percentages not provided) (Chinn, 1989). For a comparison of the major constituents of turpentine produced in the United States and other countries and the structures of the constituents, see **Table 1** and **Figure 1**, respectively. Constituents that are derived from turpentine for use in other products are listed in **Table 2.**

Table 1. Constituents and Density of Turpentines Originating in Different Countries* (order based on decreasing amount of α-pinene)

Origin	Composition (Weight Percent)					Specific Gravity (g/mL at 20 °C)
	α-Pinene	β-Pinene	Camphene	3-Carene	Limonene	
Turpentine Oil						
Greece	92-97	1-3	~1	0-1	0-2	0.860-0.865
Mexico	70-95	2-15	2-15	1-2	0-4	0.862-0.868
China	70-95	4-15	1-2	0-5	1-3	0.860-0.865
Portugal	70-85	10-20	~1	n.p.	1-4	0.860-0.870
South America	45-85	5-45	1-3	0-2	2-4	0.860-0.870
Indonesia	65-85	1-3	~1	10-18	1-3	0.865-0.870
France	65-75	20-26	~1	n.p.	1-5	0.865-0.871
Russia	40-75	4-15	1-5	0-20	0-5	0.855-0.865
Poland	40-70	2-15	~1	0-25	1-5	0.855-0.865
USA/Canada	40-65	20-35	~1	0-4	2-20	0.860-0.870
New Zealand	30-50	40-60	n.p.	n.p.	n.p.	n.p.
India	20-40	5-20	1-5	45-70	n.p.	0.850-0.865
Sulfate Turpentine						
Finland	55-70	2-6	~1	7-30	~4	0.860-0.870
Sweden	50-70	4-10	~1	15-40	1-3	0.860-0.870
USA	40-70	15-35	1-2	2-10	5-10	0.864-0.870
Russia	55-70	1-5	1-8	10-25	3-8	0.858-0.868
Steam-distilled (Wood) Turpentine						
USA	75-85	0-3	4-15	n.p.	5-15	0.860-0.875

Source: Gscheidmeier and Fleig (1996), In: *Ullmann's Encyclopedia of Industrial Chemistry*, p. 273
n.p. not provided

Figure 1. Structures of the Major Constituents of Oil of Turpentine*



3-Carene [498-15-7] | β-Pinene [127-91-3] | α-Pinene [80-56-8] | Limonene (Dipentene) [138-86-3] | Camphene [79-92-5]

*Taken from Gscheidmeier and Fleig (1996), In: *Ullmann's Encyclopedia of Industrial Chemistry*.

Table 2. U.S. Companies that Produce Products Derived from Turpentine*

Company	3-Carene	*d,l*-Limonene	*d*-Limonene	*l*-Limonene	*α*-Pinene	*β*-Pinene
Aldrich Chemical Company, Incorporated (Milwaukee, WI)					X	X
Arizona Chemical (Panama City, FL)		X	X		X	X
Bush Boake Allen Incorporated (Jacksonville, FL)		X	X		X	X
Florida Chemical Company, Incorporated (Winter Haven, FL)			X			
Hercules Incorporated, Resins Division (Brunswick, GA; Hattiesburg, MI)					X	X
Millennium Specialty Chemicals Incorporated (Jacksonville, FL)			X	X	X	X
Tecnal Corporation (Anacortes, WA)	X				X	X

* Source: SRI Directory of Chemical Producers (1997)

Saturated air contains 0.65 to 0.69% turpentine by volume (at 25°C and 760 mm Hg); the vapor density of turpentine in saturated air is 1.024 (Anonymous, 1967).

The major constituents of turpentine, particularly the reactive pinenes, determine its chemical properties. Typical reactions include isomerization, disproportionation, and polymerization catalyzed by acids, acid salts, and surfactants, or Friedel-Crafts catalysts. Reactions can also take place with halogens, hydrogen halides, and nitrosyl chloride. Moist air leads to autooxidation of *α*-pinene to sobrerol (*trans-p*-menth-6-ene-2, 8-diol) and resins. Hydrates can be formed in the presence of water. Pyrolysis yields mixtures of acyclic and monocyclic

terpenes, pyronenes, hydroterpenes, and p-cymene. Complex sulfur compounds and thiols are formed upon reaction with phosphorous pentasulfide and hydrogen sulfide (Gscheidmeier and Fleig, 1996). As the turpentine oil ages, it becomes ozonized by the formation of peroxide in the presence of air and water, and the odor and taste become more potent and unpleasant (Budavari, 1996).

2.3 Commercial Availability

Manufacturers reporting production volumes greater than 10,000 lbs. in 1998 are listed in **Table 3**.

3.0 Production Processes

According to Gscheidmeier and Fleig (1996) there are five types of turpentines, categorized by their starting material and production method. Details regarding the starting materials and processes involved in production are provided in **Table 4**.

Table 3. U.S. Companies Producing Greater Than 10,000 Pounds of Turpentine Annually

Turpentine Oil (CAS RN 8006-64-2)	
Abitibi Consolidated Snowflake Div.	Kimberly-Clark Corporation
Boise Cascade Corporation	Leaf River Forest Products
Bowater Inc.	Longview Fibre Company
Bush Boake Allen Inc.	Millennium Specialty Chemicals, Inc.
Champion International Corp.	Potlatch Corporation
Donohue Industries Inc.	Rayonier, Inc.
Florida Coast Paper Co., L.L.C.	Simpson Paper Co.
Fort James, Pennington, Inc.	Simpson Pasadena Paper Co.
Gaylord Container Corporation	Simpson Tacoma Kraft Co.
Georgia-Pacific Corporation	Smurfit Stone Container Corp.
Georgia-Pacific Fluff Pulp Operations	St. Laurent Paper Products Corp.
Gilman Paper Co.	Stone Container Corp.
Hercules, Inc.	Tenneco Packaging
Inland Eastex	Union Camp Corporation
Inland Paperboard and Packaging, Inc.	Westvaco Corp.
International Paper Company	Weyerhaesuer Co.
Jefferson Smurfit Corporation	
Turpentine (CAS RN 9005-90-7)	
Akzo Nobel Coatings Inc.	Millennium Specialty Chemicals, Inc.
Fort James	Westvaco Corp.
Gaylord Container Corp.	Weyerhaeuser Co.

Source: U.S. EPA (2001): 1998 Non-confidential IUR Company/Chemical Records.

For the purposes of this report, "turpentine oil" will refer to the pure essential plant oil obtained from resin (balsam) of conifers of the genus *Pinus*. Turpentine oil is the total distillate only and

not formulations after extraction of certain components (e.g., pinene) (Gscheidmeier and Fleig, 1996). "Turpentine" will be used to refer to the generic sulfate turpentine or to reflect a lack of specificity on the part of the authors of the original papers.

Production of crude sulfate turpentine is performed at the large paper producers that use the kraft wood pulping process. When kraft pulping is carried out continuously, as much as 74% of the original turpentine present in the wood can be extracted. Due to the presence of the sulfur compounds, the product has a dark color and a foul odor (Chinn, 1989). The crude sulfate turpentine is sold to other companies for further distillation and purification to obtain constituent products. In the United States, several companies derive terpenes from sulfate turpentine (see **Table 2**) (Chinn, 1989).

Sulfite turpentine is a byproduct of pulp production by the sulfite process and consists of 70-90% *p*-cymene. Other compounds in sulfite turpentine include dipentene, borneol, and mono- and sesquiterpenes. When mild digestion conditions are used, significant quantities of α-pinene are produced. The production of destructively distilled wood turpentine is no longer of commercial importance (Gscheidmeier and Fleig, 1996).

4.0 Production and Import Volumes

Annual worldwide production of turpentine has been estimated at 330,000 tons and 250,000 tons for 1995 and 1998, respectively (Coppen and Hone, 1995; Plocek, 1998). Turpentine oil represents about one third of the total turpentine production; sulfate turpentine makes up the remaining two-thirds (Coppen and Hone, 1995). Production of turpentine oil peaked in the U.S. in the 1950's and has experienced a declining trend for the last 50 years. The production of turpentine oil is very labor intensive; increases in wages have made its production in the U.S. economically infeasible. Georgia is the only state in which tree balsam collection continues with production levels in the range of a few thousand tons annually (Coppen and Hone, 1995). In 1999, total domestic production of turpentine was 20.7 million gallons (~7500 tons), the lowest value reported in two decades. Only sulfate turpentine was produced in the U.S. in 1999 (Howard, 2001).

Table 5 provides additional information on the production of turpentine, by type, in the United States. Worldwide, 55% of the pulping factories use the sulfate process, generating sulfate turpentine as a byproduct (Gscheidmeier and Fleig, 1996). Indeed, the predominant form of turpentine currently produced in the U.S. is sulfate turpentine. By 1989, Akzo Chemicals Incorporated (Baxley, GA) was the only company listed as a producer of turpentine oil in the United States, with production levels reported at less than 200 thousand gallons (7.2 lb/gal = 720 tons). Steam-distilled wood turpentine is also expensive to produce due to costs associated with specialized equipment. In 1989, the only U.S. company producing steam-distilled wood turpentine was Hercules Incorporated (Chinn, 1989).

Table 4. Types of Turpentine, Starting Materials, Production Processes, and Yields

Type of Turpentine	Starting Material	Production Process	Turpentine Yield (% Yield)
Turpentine oil (production peaked in the U.S. in the 1950s)	Wood resin or crude balsam from conifers, especially of the pine family	Crude balsam is freed of foreign material by filtration through finely meshed sieve. The product is washed with hot water and distilled ("normal" or gentle steam) to remove "irritants."	~1.5 kg per Scots pine; 2.5-4 kg per black pine; ~0.6 kg per spruce; Central American species may produce >5 kg balsam/tree. (13-25%)
Steam-distilled wood turpentine (wood turpentine; produced primarily in the 1960s and 1970s)	Finely chopped stump wood (usually aged)	Steam distillation or extraction with solvents (naphtha or chlorohydrocarbons), under pressure if required. This extract is then subjected to either fractional- or steam-distillation. The fraction with the lowest boiling point is identified as wood turpentine.	2.2 wt % from yellow pine stumps at least 8 yr old; 0.75 wt % from Scots pine stumps (age n.p.)
Sulfate turpentine (byproduct of kraft pulp production; produced from 1970s to present)	Debarked, finely chopped wood	Wood heated to 150-180°C in an aqueous digestion liquor (NaOH, Na_2S, Na_2CO_3 with small amounts of Na_2SO_4, Na_2SO_3 and $Na_2S_2O_3$) in large pressure vessels at 7-13 bar, for 1-5 h. The crude sulfate turpentine is condensed from the vapors of wood digestion. Sulfur compounds (methanethiol, dimethyl sulfide) are oxidized with sodium hypochlorite solution at 60°C to less volatile sulfonic acids, sulfoxides, or sulfones. These are removed by a variety of methods. This product has a characteristic musty odor.	6-16 kg/T pulp pine; 2-3 kg/T fir; 2 kg/T spruce
Sulfite turpentine (byproduct of pulp production by sulfite process; production dates n.p.)	Very small wood chips	Wood chips are impregnated with the pulping liquor ($Ca(HSO_3)_2$ and SO_2 (pH 1.5-2) in acid bisulfite pulping; $Mg(HSO_3)_2$ (pH 4) in bisulfite pulping; and Na_2SO_3, $NaHCO_3$, $(NH4)_2SO_3$, and NH_3 (pH 7-9) in neutral sulfite process) at increasing temperatures to 110°C, alternating between reduced and increased pressure. Chips are digested at 125-160°C at 7-13 bar for 6-10 h. After releasing the waste gas, the crude oil (floating on pulping liquor) is removed and neutralized with NaOH or lime and distilled to an almost colorless sulfite turpentine with a boiling point of 176-180°C.	~0.3-1 kg/T pulp
Destructively distilled wood turpentine	Pinewoods, primarily pine stumps	Obtained by the dry distillation of resin-rich pinewood followed by purification.	n.p.

Abbreviations: h = hour(s); kg = kilogram; n.p. = not provided; T = ton; wt = weight; wt% = percent weight; yr = year(s)
Source: Gscheidmeier and Fleig (1996), In: *Ullmann's Encyclopedia of Industrial Chemistry*

Table 5. Proportions (%) of Different Types of Turpentines Produced in the United States*

Type of Turpentine	1950	1960	1970	1980	1990
Turpentine Oil	38.4	19.7	4.5	1.0	0.1
Steam-Distilled (Wood) Turpentine	33.5	26.9	16.4	8.0	2.9
Sulfate Turpentine	27.4	53.4	79.1	91.0	97.0
Destructively-Distilled Wood Turpentine	0.7	0	0	0	0

*Source: Gscheidmeier and Fleig (1996), In: *Ullmann's Encyclopedia of Industrial Chemistry*.

5.0 Uses

Turpentine has long been associated with "naval stores," an archaic term still in use today that refers to rosin, turpentine, tall oil, and pitch, all produced from pine trees or pine stumps. These products were used in the past for sealing and caulking of wooden naval ships, hence the term "naval stores" (Harima Chemicals, Inc., 1997). According to the Naval Stores Act of 1923, "…'gum of spirits of turpentine' means spirits of turpentine made from gum (oleoresin) from a living tree and 'gum rosin' means rosin remaining after the distillation of gum spirits of turpentine" (Runyan, 1992). The Department of Defense and NATO still use the term "naval stores" to refer to "any articles or commodities used by a naval ship or station, such as equipment, consumable supplies, clothing, petroleum, oils, and lubricants, medical supplies, and ammunition" (U.S. Military, 2001).

Turpentine, formerly the most widely used paint thinner, is still employed in paints (both household and artist) as well as in other coatings (ACGIH, 1991; Gscheidmeier and Fleig, 1996). Historically, turpentine was used to dilute printer's ink (Naval Historical Center, 1998). Turpentine has also been used in the offset printing industry outside of the United States (Holmberg et al., 1982). The use of turpentine has diminished recently due to the availability of less expensive petroleum-based solvents (Cronin, 1979; Gscheidmeier and Fleig, 1996). Currently, turpentine continues to be used as a solvent or diluent for various products, such as natural or modified binders, resins, including alkyd resins, oils, paints, and polishes. In oil-based paint and coating formulations, peroxidation of the terpenes in turpentine accelerates the drying of oils and other film formers. Reports from the United Kingdom indicate local use of turpentine in the pottery and ceramic coating industry (Cronin, 1979; Lear et al., 1996). Turpentine is occasionally present in furniture and black shoe polishes (ACGIH, 1991, Gscheidmeier and Fleig, 1996) and continues to be used in varnishes in home crafts to finish wood (Bertelsen, 1997). Turpentine-use sites on the Internet appear to have been developed by hobbyists and possibly small companies (Fine Organics, 2000; Celtic Knot, undated). When mixed with beeswax and linseed oil, turpentine can be used both as metal polish and as a protective coating (Google Search, 2001). No statistics were found for the current use of turpentine in paints or varnishes in the United States.

Although the volume of turpentine used for metal cleaning cannot be determined, turpentine is currently being used as such to prepare metals for painting or other coatings (Fine Organics, 2000). Its use as a metal cleaner may be increasing due to the restrictions on other metal cleaners, such as CFC-113 and methyl formate, that are less environmentally benign than turpentine. Terpenes, derivatives of turpentine, are being used at one Motorola plant to clean circuit boards (Irwin, 1997). Higher-boiling turpentine fractions that contain large quantities of 3-carene are used in the forestry products industry as pesticides. Sulfate turpentine, when overproduced, has been used as a fuel in some countries (Gscheidmeier and Fleig, 1996).

Even though its use as a solvent has decreased, turpentine has attracted tremendous interest and use as a raw material for the chemical industry (Gscheidmeier and Fleig, 1996). In 1988, 209 million pounds of derivatives were produced from sulfate wood turpentine (Chinn, 1989). Terpenes and other compounds derived from turpentine are used as raw materials or submaterials for products such as tires, plastics, adhesives, flavors and fragrances, cosmetics, paints, and pharmaceuticals (Yasuhara Chemicals, undated). Separation by process-scale chromatography can yield α-pinene (purity up to 99%) and β-pinene, 3-carene, and monocyclics (α-terpinene, limonene, and phellandrene). Steam distillation of the residue separates out the higher boiling fractions (terpene alcohols, sesquiterpenes, and diterpenes) (Gscheidmeier and Fleig, 1996). Turpentine derivatives are essential ingredients in the manufacture of fragrance chemicals. The value of turpentine reflects about 25% of the value of all aroma chemicals produced both for sale and for internal use each year (Plocek, 1998). Some of the chemicals derived from turpentine along with their uses are listed in **Table 6**.

Turpentine oil has been used in the preparation of pharmaceutical and cosmetic products. Twenty to 30 years ago, turpentine was used in hairdressing preparations, but there is no evidence of its continued use in hair salons today (NIEHS, 1998). Although not representative of the entire industry, a major producer of cosmetics and household products in Germany indicated an increased use of turpentine oil during 1996 to 1997 compared to 1990-1995 (Treudler et al., 2000).

In the past, turpentine oil was used medicinally both externally and internally. A clear distinction was made between turpentine oil and the steam-distilled wood turpentine, with only the former accepted for use medicinally. Externally, turpentine oil was used in liniments as a stimulant and counterirritant. Turpentine to be taken orally was “rectified” by reacting it with sodium hydroxide. Most of the original oil was distilled off the sodium hydroxide/turpentine mixture, and then dried with either anhydrous calcium chloride or anhydrous sodium sulfate. Rectified turpentine was used in human and veterinary practice as a stimulant diuretic, anthelmintic, carminative, and expectorant (Martin and Cook, 1961; Baxter, 2001).

Turpentine oil is currently used in massage oils and aromatherapy products (ATL Canada, 2000; Burfield, 2000), and has been used in traditional medicine to treat problems of the respiratory tract (Pifferi, 1994). Vicks VapoRub, a topical ointment used to treat symptoms associated with the common cold, contains turpentine at a concentration of 4.7% w/w (Malahyde Information

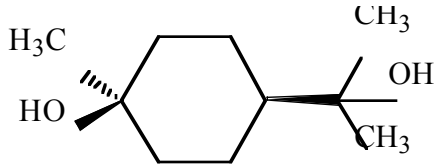
Table 6. Chemicals Derived from Turpentine and Their Uses*

Chemical	Use
Anethole	Flavor ingredient, insect attractant
Camphor	Pharmaceutical, plasticizer, fragrance
3-Carene	Pesticide
p-Cymene	Disinfectant, solvent
Esters of pinic acid	Plasticizer
Estragole	Flavor ingredient and perfume fragrance
Geraniol	Fragrance
Isobornyl acrylate	Varnish resin
Isobornyl phenols	Antioxidant
d,*l*-Limonene (Dipentene)	Cleaning agent, solvent, flavor and fragrance additive
Linalool	Fragrance
p-Menthane	Solvent
Menthol	Pharmaceutical
Nerol	Fragrance
Pinane	Polymerization accelerator for rubber
Pine oil	Solvent, textile auxiliaries, flotation aids, cleaning and disinfecting products
α-and β-Pinene	Fragrance
β-Pinene polymers	Resin
Terpene ethers	Traction fluids
Terpene halides	Pesticide
Terpene-maleate resins	Varnish resin
Terpene phenols	Varnish resin, melt adhesive
Terpene polymers	Melt adhesive
Terpineol	Disinfectant, textile auxiliaries, fragrance
Terpenes containing sulfur	Lubricating oil additives
α-Pinene	Pinene
Toxaphene	Pesticide
Others	Fuel additives, oil field chemicals, fragrances

* Sources: Gscheidmeier and Fleig (1996); NTP (TR-347, 1990)

Systems, 1998; OTC Service, undated; Xrefer, 2001). It has been claimed that turpentine has anti-inflammatory and analeptic properties, with high affinity for the respiratory tract and low toxicity.

Turpentine was used to manufacture the drug terpin hydrate (terpinol hydrate) by treating it with sulfuric acid at 20-30°C for three to six days while stirring and blowing air through the mixture (Martin and Cook, 1961). The *cis*-form of terpin hydrate was used as an expectorant, especially in the treatment of bronchitis (Martin and Cook, 1961; Budavari, 1996).



cis-Terpin

6.0 Environmental Occurrence and Persistence

Turpentine is a natural product and is completely biodegradable. Below the solubility limits, turpentine does not represent a hazard to biological wastewater-treatment plants (Gscheidmeier and Fleig, 1996). However, the biological and chemical oxygen demand for turpentine is exceptionally high (Irwin, 1997) and therefore effluent discharges are regulated (40 CFR 454.22 and 40 CFR 454.32). Environmental releases of turpentine may occur at production facilities where faulty equipment or spills occur. Facilities are required to use best management practices (BMP) to reduce the amount of turpentine released to the air during turpentine production processes (40 CFR 63.446; 40 CFR 430.3).

The Clean Air Act of 1990 did not classify terpenes, constituents of turpentine, as air polluting substances. Turpentine released into the environment is completely degraded by natural processes within a few days. The rate of degradation depends on the concentration of turpentine, temperature, availability of air, and presence of bacteria. Turpentine has been ranked as having zero potential as an ozone depleting substance or for global warming (Gscheidmeier and Fleig, 1996).

7.0 Human Exposure

Turpentine is derived primarily from trees of the *Pinus* ssp. Volatile terpenes, components of turpentine, are emitted into the atmosphere by these trees, especially in the summer. Annual worldwide biogenic production of terpenes has been estimated at 10^9 tons. Exposures to these terpenes would occur by simply walking through a pine forest (Gscheidmeier and Fleig, 1996).

According to the Occupational Safety and Health Administration (OSHA, 1999), exposures to turpentine occur from the manufacture of turpentine oil and the rosin remaining after removal of the turpentine oil and its primary industrial uses as an ingredient (e.g. flavoring agent), solvent, industrial coatings, and starting material for other compounds. In addition, exposures can occur during pulp and paper processes. The general public may be exposed through foods, personal care products, household products, and external and internal medications. Hobbyists may be exposed to turpentine through the use of paints and varnishes. The industries with the highest percentage of exposure to turpentine, according to the 1981-1983 NIOSH Occupational Exposure Survey, are listed in **Table 7**.

Inhalation and dermal exposures to turpentine represent industrial hygiene concerns (Santodonato et al., 1985). Until the 1940s, the major use of turpentine was as a solvent in paint production. Since that time, the use of turpentine as a solvent has been almost entirely replaced by petroleum solvents (Stonecipher, 1955, 1969; both cited by Santodonato, 1985). Although still used by professional painters and private individuals as a solvent, thinner, cleaning agent, and as a storage medium for paints, varnishes and equipment, the predominant use of turpentine is as a source of the individual components (α-pinene, β-pinene, and ρ-menthadienes) and pine oil (Santodonato, 1985). Current exposures during painting appear to be limited to special applications such as in art, ceramics, and ship painting (Lear et al., 1996; USJobBoard, 2000). The greatest populations at risk for dermal exposure to turpentine are artists, cabinetmakers, carpenters, painters, and

Table 7. Industries with the Largest Percentage of Workers Exposed to Turpentine According to the 1981-1983 NIOSH Occupational Exposure Survey*

Standard Industrial Classification Code	Industry	Exposed Workers by Facility Employment Size (Percentage of Total Workers Exposed to Turpentine)		
		Small (1–99)	Medium (100–499)	Large (500 +)
16	Heavy Construction Contractors	0	9,807 (7.2%)	0
17	Special Trade Contractors	19,963 (2.2%)	0	-----
27	Printing and Publishing	5,354 (1.8%)	9,218 (4.2%)	1,469 (1.0%)
34	Fabricated Metal Products	1,654 (0.4%)	24,226 (6.3%)	4,177 (2.1%)
35	Machinery, Except Electrical	16,743 (4.0%)	23,468 (6.9%)	48,682 (9.4%)
36	Electrical and Electronic Equipment	18,461 (12.9%)	56,155 (17.1%)	51,724 (10.4%)
37	Transportation Equipment	0	5,215 (3.3%)	11,257 (1.9%)
38	Instruments and Related Products	8,360 (12.6%)	17,667 (19.2%)	8,709 (5.1%)
48	Communication	7,710 (4.7%)	1,465 (2.0%)	2,380 (49.9%)
72	Personal Services	15,117 (5.4%)	0	0
73	Business Services	11,070 (4.1%)	1,023 (0.4%)	2,114 (1.5%)
76	Miscellaneous Repair Services	9,724 (8.6%)	1,480 (6.7%)	-----

* The industries with the highest numbers of exposed workers were chosen for inclusion if the numbers of exposed workers in that industry comprised more than 2% of the total number of exposed workers in all 32 industries that reported exposures. Source: Pedersen et al. (2001).

shoemakers (CCOHS, 1997). Turpentine has also been used in spray finishing operations, where it is sprayed on the surfaces to be treated or cleaned, leading to potential exposures (29 CFR 1910.94). Instructions on newer residential oil-based paint formulations actually caution users not to thin the paint with turpentine, but instead use mineral spirits. Dermal exposure among the general public may occur from the use of massage oils (ATL Canada, 2000; Burfield, 2000).

Inhalation exposure can occur among sawmill workers (when monoterpenes are liberated during sawing or processing of fresh wood), artists (canvas or ceramics), varnishers or furniture finishers, or other specialized painters (Lear et al., 1996). Individuals working in pulp and paper facilities (Hogstedt, 1990), in the manufacture of turpentine, or in the isolation of individual constituents from turpentine have the potential to be exposed to turpentine. However, such exposures are limited as most kraft paper and turpentine production processes are conducted in closed systems to both prevent product loss and to regulate process conditions.

8.0 Regulatory Status

U.S. government regulations promulgated by the Food and Drug Administration (FDA), the U.S. Environmental Protection Agency (EPA), OSHA, and the U.S. Department of Transportation

(DOT) pertaining to turpentine are summarized in **Table 8**. Other regulatory activities of concern are summarized in the following text.

In a broad effort to remove ineffectual ingredients in non-prescription drugs, the FDA began the review of all non-prescription drugs in 1972 (FOI Services, 1989, 1990). In 1987, turpentine oil was considered by the FDA to be a "nonmonograph" ingredient in cough suppressant formulations and not to be used as such without FDA approval (Washington Drug Letter, 1987). Furthermore, in 1989, the FDA ruled that only one ingredient (guaifenesin) was effective as a cough expectorant. All formulations containing turpentine oil had to be reformulated within 12 months, or have sales halted (FOI Services, 1989). Turpentine oil was also banned for use in nasal decongestant medications unless the manufacturer could prove to the FDA that it was safe and effective as a nasal decongestant (FOI Services, 1990).

In 1992, the FDA proposed banning 415 ingredients in over-the-counter medications because they were not shown to be safe and effective for their stated claims (FDA, 1992). Turpentine was listed as one of the ingredients to be banned for treatment of fever blisters, cold sores, insect bites and stings, menstrual pain, and treatment for poison ivy, oak, and sumac. Most of the ingredients had been in use prior to 1962, when a change in the law required drug manufacturers to submit proof of effectiveness for new drug products. No further information on this action was found.

In 1996, the U.S. EPA proposed rules to control the emissions of hazardous air pollutants (HAPs) from waste-burning incinerators, cement kilns, and lightweight aggregate kilns. The final rule (40 CFR Parts 63, 261, and 270) discussed a "comparable fuel exclusion" for fuels derived from hazardous wastes. The hazardous waste-derived fuels were required to meet similar specifications as comparable fossil fuels in regards to concentrations of hazardous constituents and physical properties affecting burning. To better categorize fuels, EPA sought to establish benchmark fuels for guidance. The EPA received a comment that turpentine should be listed as a benchmark fuel since it has a very high British thermal unit value and is used as a fuel. However, EPA rejected turpentine as such because it is not widely used as a commercial fuel and there are no ASTM standards that would specify the minimum properties that must be met in order to qualify it as a fuel (U.S. EPA, 1998).

9.0 Toxicological Data

9.1 General Toxicology

9.1.1 Human Data

Many of the human toxicity studies were performed in the early 1900s when the composition of turpentine differed from the products used today. In the Unites States, turpentine oil was the predominant form of turpentine used in the first half of the 1900s, whereas steam-distilled wood turpentine was primarily produced from the 1960s until the 1970s when sulfate turpentine emerged as the major form of turpentine produced (Gscheidmeier and Fleig, 1996). In addition,

Table 8. Federal Regulations Relevant to Turpentine

Reference		Summary of Regulation
FDA	21 CFR 172.510	Turpentine as derived from *Pinus palustris* Mill. and other *Pinus* spp. and which yield terpene oils exclusively, is listed as a natural flavoring substance.
	21 CFR 175.105	Turpentine is listed as a substance allowed in adhesives for food packaging.
	21 CFR 178.3930	Terpene resins consisting of hydrogenated polymers of terpene hydrocarbons obtained from sulfate turpentine or terpene resins consisting of polymers of β-pinene, and meeting specific criteria, may be used in polypropylene or polyolefin films, respectively, intended for use in contact with food.
	21 CFR 310.545	Turpentine spirits is listed as an active ingredient in drug products offered in over-the-counter formulations including nasal decongestant drug products; expectorant drug products; fever blister and cold sore treatment drug products; insect bite and sting drug products (external application for treatment and protection); and poison ivy, poison oak, and poison sumac drug products (external application for treatment and protection). Venice turpentine[2] is used as an oral menstrual drug product. Turpentine spirits (rectified) is used as anorectal counterirritant drug product.
OSHA	29 CFR 1910.94	In regard to spray finishing operations when organic or inorganic materials are dispersed on surfaces to be coated, treated, or cleaned, there is a requirement for the total air volume exhausted through the spray booth to be sufficient to dilute the solvent vapor to at least 25% of the lower explosive limit (LEL) of the solvent being sprayed. This CFR provides a formula to determine the volume of air in cubic feet necessary to dilute the vapor from 1 gallon of solvent to 25% of the LEL.
	29 CFR 1910, Subpart Z	The permissible exposure limit (PEL), as an 8-hour time weighted average, for turpentine is 100 ppm or 560 mg/m^3 as measured in the breathing-zone air samples.
	29 CFR 1915, Subpart Z	The PEL, as an 8-hour time weighted average, for turpentine in shipyards is 100 ppm or 560 mg/m^3.
	29 CFR 1926.55	The PEL, as an 8-hour time weighted average, for turpentine in construction is 100 ppm or 560 mg/m^3 at construction sites.
U.S. EPA	40 CFR 454, Subparts B and C	Pollutant limitations from facilities that manufacture gum rosin and turpentine are provided. **Gum Rosin and Turpentine**: The biochemical oxygen demand in effluent is 1.42 kg/Mg for the maximum discharge in any one day and 0.755 kg/Mg for the maximum average daily value over 30 consecutive days. The total suspended non-filterable solids in effluent is 0.077 kg/Mg for the maximum discharge in any one day and 0.026 kg/Mg for the maximum average daily value over 30 consecutive days. The pH range is 6.0 to 9.0. **Wood Rosin, Turpentine, and Pine Oil**: The biochemical oxygen demand in effluent is 2.08 kg/Mg for the maximum discharge in any one day and 1.10 kg/Mg for the maximum average daily value over 30 consecutive days. The total suspended non-filterable solids in effluent is 1.38 kg/Mg for the maximum discharge in any one day and 0.475 kg/Mg for the maximum average daily value over 30 consecutive days. The pH range is 6.0 to 9.0.
	40 CFR 63.446	Set forth the required equipment systems in the pulping process to reduce the loss of hazardous air Pollutants (HAPs) released from the digester system, the turpentine recovery system, and evaporator system during the kraft pulping process.
	40 CFR 430.3	Stipulates and identifies the BMP that paper mills must use to prevent leaks or spills of spent pulping liquors, soap, and turpentine.
	40 CFR 455, Table 10	Activated carbon in the appropriate pollution control technology to remove turpentine from contaminated wastewater when it is used as a pesticide ingredient.
DOT	49 CFR 172.101	Listed in the hazardous materials table.

[2] Venice turpentine is derived specifically from larch (*Pinus larix*) trees. The oleoresin is perfectly clear and free from impurities once strained through a course haircloth. The name is derived from the point of origin (Venice) (Grieve, undated).

the distillation process was not as well controlled as it is presently, resulting in formulations whose constituents differed from batch to batch more than would be expected using current production methods.

9.1.1.1 Acute Effects

Acute toxicity values in humans for turpentine are presented in **Table 9**.

The mean oral lethal dose of turpentine is reported to range from 15 mL (13 g; 0.095 mol) to 150 mL (129 g; 0.947 mol) (Deichmann and Gerarde, 1964; cited by Anonymous, 1967; Grapel, 1901; Stanwell, 1901; Maitland, 1931; all cited by ACGIH, 1991; McGuigan, 1985; cited by Lewander and Aleguas, 1998). The minimum lethal dose for children has been estimated at 14 g (~16 mL; 0.10 mol) (age not provided) (Jill et al., 1975; cited by Lewander and Aleguas, 1998). Ingestion of turpentine usually results in gastrointestinal (GI) tract irritation and central nervous system (CNS) depression within two to three hours. These effects generally subside within 12 hours except in severe exposure cases. Signs and symptoms of turpentine poisoning include nausea, vomiting, diarrhea, weakness, somnolence, or agitation (Lewander and Aleguas, 1998) and in glucosuria, hematuria, albuminuria, and anuria (Chapman, 1941). Inhalation of turpentine causes an irritation of the mucus membranes of the nose and upper respiratory tract, cough, bronchial inflammation, salivation, headache, dizziness, labored breathing nausea and unconsciousness (Anonymous, 1967; ICSC, 1990). Labored breathing may not become apparent until several hours after the exposure and may be aggravated by physical activity (ICSC, 1990). Chemical pneumonitis can result from aspiration, and is associated with pathognomonic dyspnea, acute pulmonary edema, and cyanosis. Turpentine acts as CNS depressant resulting in the loss of reflexes and finally coma (Chapman, 1941). Death may occur as a result of extreme exposures (typically inhalation or ingestion) (ICSC, 1990). Alcohol consumption is reported to enhance the harmful effects of ingested or inhaled turpentine (ICSC, 1990).

Human volunteers were experimentally exposed to turpentine vapors (Swedish turpentine, Alcro Beckers) at the Swedish occupational exposure limit of 450 mg/m3 (75 ppm) for two hours. The volunteers were asked to rate their symptoms before, during, and after the exposures, based on their level of perceived discomfort, using a survey of ten questions. The questions covered irritative symptoms (discomfort of the eyes, nose, throat and airways) and CNS effects (headache, fatigue, sickness feeling, dizziness, intoxication, difficulty breathing, and smell of solvent). During the exposures to turpentine, the volunteers noted discomfort to the throat or airways. No significant differences were found in the ratings of symptoms of the CNS after exposure to turpentine relative to control exposures. There was an increase in airway resistance in the exposed volunteers.

Exposures to 75 ppm (420 mg/m^3) turpentine vapor for three to five minutes resulted in nose and throat irritation for several volunteers; exposures to 175 ppm (975 mg/m^3) for the same exposure time was considered intolerable to the majority of volunteers. According to most volunteers, the highest concentration that could be tolerated for an eight-hour period was 100 ppm or 560 mg/m^3 (Nelson et al., 1943; cited by ACGIH, 1991). Acute exposure to turpentine for several hours at

Table 9. Acute Toxicity Values for Turpentine in Humans

Route	Sex and age	LD/LC/TC	Reference(s)
Inh	Male, adult	LC_{Lo} = 449 mg/m^3 (80.6 ppm)	RTECS (2001)
	Sex and age n.p.	TC_{Lo} = 175 ppm (975 mg/m^3)	RTECS (2001)
	Sex and age n.p.	TC_{Lo} = 6 g/m^3 (1080 ppm)	RTECS (2001)
p.o.	Male, adult	LD_{Lo} = 2.86 mL/kg (2.46 g/kg; 18.1 mmol/kg)	RTECS (2001)
	Male, adult	LD_{Lo} = 3 mg/kg (0.02 mmol/kg)	RTECS (2001)
	Sex n.p., infant	LD_{Lo} = 1748 mg/kg (12.83 mmol/kg)	RTECS (2001)
	Sex n.p., infant	TD_{Lo} = 874 mg/kg (6.42 mmol/kg)	RTECS (2001)
	Female, adult	TD_{Lo} = 560 mg/kg (4.11 mmol/kg)	RTECS (2001)
i.v.	Male, adult	TD_{Lo} = 0.071 mL/kg (61 mg/kg; 0.45 mmol/kg)	RTECS (2001)
n.p.	Male, adult	LD_{Lo}= 441 mg/kg (3.24 mmol/kg)	RTECS (2001)
n.p.	Sex and age n.p.	LD_{Lo} = 1777 mg/kg (13.04 mmol/kg)	RTECS (2001)

Abbreviations: inh = inhalation; i.v. = intravenous; $LC(D)_{Lo}$ = lowest concentration (dose) tested resulting in lethality; p.o. = per os; TC_{Lo} = lowest concentration tested resulting in adverse effects; n.p. = not provided.

750 to 1000 ppm (4180-5572 mg/m^3) resulted in ocular irritation, headache, dizziness, nausea, and tachycardia (Lehman and Flury, 1943; cited by ACGIH, 1991). NIOSH has determined that a concentration of 800 ppm (4460 mg/m^3) is immediately dangerous to life and health (Ludwig, 1994).

Intrauterine administration of turpentine as an abortifacient has resulted in peritonitis, pelvic necrosis and inflammation, pulmonary edema (Martini, 1957; Quander and Moseley, 1964; both cited by ACGIH, 1991), and acute renal failure (Gornel and Goldman, 1968; cited by McIntosh et al., 1975). Morphological lesions noted six weeks after treatment included tubular atrophy and dilatation, interstitial fibrosis, edema, and leukocytic infiltration.

9.1.1.2 Reproductive and Developmental Effects

There is some concern that occupational exposures to airborne contaminants, including turpentine, may be harmful to the developing fetus. A period of fetal sensitivity is likely to be within the first trimester, the period of organogenesis (Kuntz, 1976; cited by Irwin, 1997).

In a study of children born with cleft palates, Holmberg et al. (1982) reported on 388 Finish mothers possibly exposed to aromatic solvents during the first trimester; only 14 cases were presented in the paper. Of the 14 cases reported, one was exposed to lacquer petrol, methylene chloride, and turpentine during offset printing. The usefulness of this report is of questionable value based on the low number of exposed individuals and potential for multiple exposures.

9.1.1.3 Dermal Sensitization

Allergic dermatitis as a result of dermal exposures to turpentine has been well documented (Rudner et al., 1973), and it is apparent from the published literature that dermal exposures represent an industrial hygiene concern (Santodonato, 1985).

Turpentine oil was frequently cited as a cause of allergic occupational dermatitis in the 1950s (Lear et al., 1996). There have been several studies that have looked at patterns of turpentine reactivity. McCord (1926) reported a dramatic increase in the incidence of occupational skin disease in a group of 50 workers (industry undisclosed). In a two-month time frame, 36 cases of dermatitis were reported in a group that averaged three cases of occupational skin disease per year during the prior 12 years. The cause of the increased skin disease was attributed to a change from turpentine oil to the more economical wood turpentine (either steam distilled or destructively distilled), known to be a greater irritant (McCord, 1926).

Similar situations have been described in several other reports. Lear et al. (1996) suggested that contact sensitization was under-reported within the pottery industry. Within this specific sub-population, which included ceramic decorators, turpentine is re-emerging as a cause for allergic occupational dermatitis. Substitution of Indonesian turpentine for Portuguese turpentine was the triggering event for this study, possibly due to increased concentrations of Δ^3-carene in the Indonesian product. When the patients (n = 24) were tested for reactivity, 14 were sensitive to Indonesian turpentine and three reacted to the Portuguese product. Reactivity to specific constituents of turpentine was also considered: eight patients reacted to α-pinene; four reacted to Δ^3-carene, and two to turpentine peroxide (the form used most often for skin-patch tests). α-Pinene represents the principle component in the fine quality turpentine used by the ceramics industry (Lear et al., 1996).

A subset of 37 painters, varnishers, and lacquerers were identified for a study of occupational dermatitis (Moura et al., 1994). This group included house (14) and automobile (nine) painters, naval painters (four), ceramic painters (one), and seven users of paints, varnishes, and lacquers. Patients were patch-tested to determine the cause of the contact dermatitis. Of the 37 patients tested, eight were sensitized to turpentine. The components most likely to cause sensitization were cited as dipentene (limonene) and α-pinene (Moura et al., 1994).

Several studies have shown a decline in sensitization to turpentine since the mid-1970s in the general public and in some occupational exposures (Cronin, 1979; Lear et al., 1996). Cronin (1979) reported a low of 0.7% positive reactivity to turpentine within a primarily European population and recommended the removal of turpentine from the standard patch test. In a North American population, turpentine was shown to be among the top seven most reactive allergens tested. Six percent of the tested North American population reacted positively to turpentine; however there was some concern that the results were confounded by the irritancy properties of the 1% aqueous solution used in this population (Rudner et al., 1973).

The decline in reactivity to turpentine is presumably due to the decreased use of turpentine in paints, and its replacement with water-soluble paints and less expensive paint thinners (Moura et al., 1994; Lear et al., 1996). However, turpentine's increased use in the pottery industry and the use of pine oils in European cosmetic products have resulted in a new pattern of turpentine sensitization (Rudzki et al., 1991). Dermatitis caused by turpentine has been attributed primarily to 3-carene, a variable constituent of turpentine oil (Cronin, 1979; Gosselin et al., 1984, cited by ACGIH, 1991). More recent studies show that α-pinene, β-pinene, and *d*,*l*-limonene (dipentene) are all strong sensitizers (Romaguera et al., 1986; Moura et al., 1994). Lear et al. (1996) speculated that terpene hydroperoxide products, rather than the terpenes themselves, may be the major sensitizers in turpentine.

A more recent investigation into the rates and causes of turpentine sensitization suggests a very recent increase in turpentine sensitization. Treudler et al. (2000) reported on the findings of a large study in Europe. Patch tests were performed in 45,005 patients during the years of 1992 through 1997 using the standard series of the German Contact Dermatitis Research Group. This panel includes turpentine (10% in petrolatum, supplied by Hermal, Reinbek, Germany). The source of the turpentine preparations used for the entire study originated from Portugal. For the years 1992 through 1995, positive reactions to the Hermal turpentine preparation remained fairly consistent and ranged from 0.3% to 0.6%. However, in 1996, the rate more than tripled over the previous year (from 0.5% in 1995 to 1.7% in 1996). Furthermore, additional testing in 1997 suggest that the previous years' findings were real, as the percentage of positive responders doubled once more to 3.1%. Significantly more of the positive responders were women and older patients (> 60 years). Symptoms occurred more frequently on the face and legs rather than on the hands, as is frequently the case in occupational exposures. In addition, the analysis of the patients' occupations gave no hint of any relevant occupational exposures (pensioners, housewives, students, office clerks, or unemployed). The authors suggested that the source of the allergens might be found in liniments and creams, especially for patients with leg problems, such as dermatitis due to venous insufficiency or leg ulcers. The high rate of reactivities to fragrances, balsam of Peru, and colophony may be due to group or cross allergies. There are many compounds that have been shown to cross react with turpentine, such as ragweed, chrysanthemums, peppermint, and bergamot oil. There was also a suggestion that this increase in reactivity to turpentine may be linked to the increased use of tea tree oil. Tea tree oil is a mixture of many terpenes including 30% terpinenes and not more than 15% cineole. Allergic components of tea tree oil may include limonene, α-terpinene, and aromadendrene. These authors recommend the continued presence of turpentine on the standard patch test (Treudler et al. 2000).

Some people who had originally tested positive for skin sensitivity to turpentine oil have not reacted positive when tested years later. Twenty-eight volunteers (17 men and 11 women) who previously tested positive for sensitivity to turpentine were administered the Scandinavian Silverpatch test with turpentine peroxide (0.3% in olive oil) some two to 15 years after the first test (Lintum and Nater, 1973). Eighteen volunteers (64%) did not exhibit a second reaction. There was no correlation between individuals who had avoided contact with turpentine between tests and the lack of a second response. Other studies in which diminished sensitization was

observed on subsequent patch testing with turpentine were cited by Lintum and Nater (1973). The incidence of a negative response after an initial positive test ranged from 6% to 66% in the various studies reviewed.

Different methods of turpentine production, source of the pine trees used to derive the turpentine, and season of harvest, will yield formulations with different chemical characteristics and thus presumably different toxicity profiles (McCord, 1926; Cronin, 1979). Sulfate wood turpentine produced in Sweden, Finland, and Russia contains high concentrations of 3-carene (30-40%). In balsam turpentine spirits produced in the United States and Southern Europe, concentrations of Δ^3-carene are much lower or negligible. In 1939, it was first shown that the greater dermal toxicity seen in Swedish versus French painters was due to the presence of a hydroperoxide product of 3-carene in turpentine (Hellerström, 1939 cited by Cronin, 1979). In one study, sensitivity to Indonesian turpentine was greater than sensitivity to Portuguese turpentine. Differences in the major constituents of these two turpentines were noted, with Indonesian turpentine containing greater than 15% Δ^3-carene and the Portuguese product containing greater than 15% β-pinene (Lear et al., 1996). A recent study showed that a brand of turpentine produced in Finland (Oulu 1) contained as much as 53% *d*-Δ^3-carene (Kasanen et al., 1999). In addition to 3-carene, steam-distilled turpentine may contain other chemicals that are dermal irritants. For example, the liquid condensate of wood turpentine from the still was reported to contain acetic acid (~4%), methyl alcohol (2 to 4%), formic acid (1%), formaldehyde (1 to 2%), furfuraldehyde (1%), and methal acetate (2 to 4%). These chemicals may cause dermal irritation (McCord, 1926).

Toxicity may also be attributed to breakdown products of turpentine. Crude sulfate turpentine can break down in the environment to methyl mercaptan (Chandler et al., 1997). The reaction of the monoterpene constituents of turpentine (unsaturated hydrocarbons) with typical oxidants in indoor air, such as ozone, can form potent substances (e.g., formaldehyde) or radicals that may cause sensory irritation even if the monoterpenes are at acceptable levels (Kasanen et al., 1999).

9.1.1.4 Chronic Effects

There are a limited number of studies investigating the chronic effects associated with occupational exposures to turpentine. Chronic toxic effects associated with occupational exposures to turpentine include cerebral atrophy, behavioral changes, anemia and bone marrow damage, glomerulonephritis and dermatitis (Sandmeyer, 1981; cited by Santodonato, 1985). Hematologic effects have also been noted. Early studies of painters in the United States, France, and Australia resulted in conflicting results regarding kidney toxicity (Nicholl, 1911; Heim et al., 1923; Fairley et al, 1934; all cited by Chapman, 1941), possibly due to combined exposures with lead-based paints. Urinary disturbances, albuminuria, and urinary casts (uncharacterized) were observed in workers exposed to paint and varnishes (Nicholl et al., 1911; cited by Chapman, 1941). In Europe and Australia, no kidney problems were reported in painters exposed to turpentine and zinc, but urinary abnormalities were reported in painters exposed to turpentine and lead (Heim et al., 1923). Any renal damage associated with long term occupational exposures to turpentine have been transient and completely reversible (Gleason et al., 1963; cited by

McIntosh et al., 1975). Pedersen and Rasmussen (1982) reported a decrease in leukocyte counts among 64 solvent-exposed (turpentine, toluene, and xylene) workers, compared with unexposed volunteers.

9.1.1.5 Epidemiology

In 1980 IARC (International Agency for Research on Cancer) made the following statement regarding turpentine:

> Epidemiological data are not sufficient to make a definitive assessment of carcinogenic risk of employment in the paper or pulp mill industries. Several studies suggest an increased risk of lymphoproliferative neoplasms, particularly Hodgkin's disease and perhaps leukemia may be linked to employment in paper and pulp industries.

During the 1980s, a number of epidemiological studies were performed, including cohort, proportionate mortality ratio (PMR), and case control studies. Hogstedt (1990) reviewed these studies. Kauppinen et al. (1986; cited by Irwin et al., 1997) performed a nested case control analysis that was not included in the review by Hogstedt.

In four studies (Kauppinen et al., 1986; cited by Irwin, 1997; Jäppinen et al., 1987; Carstensen, 1987; Thorén et al., 1989; the latter three cited by Hogstedt, 1990), the excess risk of lung cancer associated with working in pulp and paper related industries was considered. In two of the studies, a significant association was described between exposures to terpenes and other heating products of coniferous wood and the risk for lung cancer. This was especially noticeable for employees exposed for more than five years (odds ratio [OR] = 9.71, confidence interval [CI] not provided, $p < 0.05$) (Kauppinen et al., 1986; cited by Irwin, 1997) and in boardmill workers exposed for greater than 20 years (78 observed vs. 62.6 expected lung cancers) (Jäppinen et al., 1987; cited by Hogstedt, 1990). Kauppinen et al. (1986; cited by Irwin, 1997) relied on work histories and job exposure matrices to estimate exposures. There were no industrial hygiene data available for the Jäppinen study, though the authors of this study stratified a random sample of the study population according to smoking habits, so it was unlikely that the lung cancers were due to smoking behavior. Thorén et al. (1989; cited by Hogstedt, 1990) observed a slight increase in risk of lung cancer associated with employment in soft papermill plants in a case referent study. However, the increased risk (OR = 1.6, 95% CI 0.7-3.2) was based on only six exposures. One study of standard incidence ratios (SIRs) (Carstensen, 1987; cited by Hogstedt, 1990) reports a substantially decreased SIR for lung cancer from cellulose, papermill, paper, and paperboard workers. This study was adjusted for smoking.

Lymphoproliferative diseases were considered in five studies. In some cases, the distinction between processing processes (sulfate vs. sulfite) was attempted. In four of the five studies, increases in lymphoproliferative diseases were reported. An increase in lymphosarcomas associated with greater than 20 years in the sulfate pulping industry was reported in a cohort

study by Robinson et al. (1986; cited by Hogstedt, 1990). Likewise, Milham (1983; cited by Hogstedt, 1990), deriving their information from Washington State death records, reported increased proportional mortality ratios (PMR) for lymphatic and hematopoietic tissues, particularly Hodgkin's disease (194, n = 17), multiple myeloma (182, n = 19) and leukemia (126, n = 38). When the study was expanded to include the trade industry, the increased risk of non-Hodgkin's lymphosarcoma appeared to be associated primarily with the sulfite pulping industry, while Hodgkin's disease was associated more with the sulfate pulping industry (Milham and Demers, 1984; cited by Hogstedt, 1990). Schwartz (1988; cited by Hogstedt, 1990) also reported an increased PMR (12 observed vs. 7.5 expected) for leukemia in pulp, paper and paperboard mill workers.

Possible associations between cancers of the digestive organs and employment in the pulp, paper and associated industries have also been studied. Two cohort studies (Robinson et al., 1986; Jäppinen et al. 1987; both cited by Hogstedt, 1990) gave conflicting results. While Robinson et al. report an excess risk of stomach cancer associated with the sulfite pulp process (9 observed vs. 5.1 expected), Jäppinen et al. indicated an overall deficit of tumors of the digestive organs, including stomach. Milham and Demers (1984; cited by Hogstedt, 1990) indicated that the increase in stomach cancer was associated with the sulfite and sulfate processing plants but not with the papermills in a PMR analysis. Finally, in a case-control study, deaths for stomach cancer were associated with paper mills using the sulfite process (relative risk = 2.7, n = 13) (Wingren et al., 1985; cited by Hogstedt, 1990).

Epidemiological studies of pulp and paper workers are confounded by other possible chemical exposures, such as hydrogen sulfide, various other organic sulfides and mercaptans, sodium hydroxide mist, methanol, ethanol, sulfuric acid, furfural, hydroxymethylfurfural, cymene, acetic acid, formic acid, gluconic acid, aldonic acid, hydrogen peroxide, and complex chlorinated compounds (e.g., pentachlorophenol). Without job-exposure matrices in the pulp and paper industries, it is difficult to pinpoint exposure to specific chemicals and the corresponding risks of developing cancers.

More recently, a multicenter case-control study was performed to investigate the impact of parental exposures to specific chemicals on the incidence of neuroblastoma in their children. Children (538, aged 19 years) with newly diagnosed and confirmed neuroblastoma during the period of 1992 to 1994 were compared to age-matched controls (504). Although maternal exposures to most chemicals were not associated with neuroblastoma in their offspring, paternal exposures to turpentine were associated with an increased incidence of the tumor (OR 10.4; 95% CI 2.4, 44.8) (De Roos et al., 2001).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

Turpentine is readily absorbed through the GI and respiratory tracts and skin (Baxter, 2001). The solubility of terpenes in human blood is high. Blood:air partition coefficients are 15, 23, and 32 for α-pinene, β-pinene, and 3-carene, respectively (Filipsson, 1996). This suggests that absorption by the lungs is very efficient. When human volunteers inhaled turpentine (450 mg/m^3,

80.7 ppm) while performing light activity, the relative uptakes of α-pinene, β-pinene, and 3-carene averaged 62%, 66%, and 68 %, respectively (Filipsson, 1996). Concentrations of α-pinene, β-pinene, and 3-carene in the blood peaked two hours after administration at about 9.5, 10.25, and 2.5 µmol/L, respectively.

Since turpentine is a lipophilic substance, it accumulates in fatty tissues. In rats, the highest concentrations of inhaled turpentine were found in the spleen, kidneys, brain, and peripheral and perinephric fat (Sperling et al., 1969; cited by ACGIH, 1991; Savolainen and Pfäffli, 1978). In rats exposed to turpentine at 300 ppm (1670 mg/m^3), a concentration three times that of the standards established by OSHA and NIOSH (100 ppm or 560 mg/m^3), α-pinene accumulated in brain and perinephric fat throughout the eight-week exposure period (Savolainen and Pfäffli, 1978) (see **Table 8**). No studies that determined the half-life of turpentine in biological systems were found.

Elimination of turpentine and its metabolites is primarily through the urinary tract (Lewander and Aleguas, 1998). When turpentine was inhaled by human volunteers (450 mg/m^3, 80.7 ppm), only 2 - 8% of the amount absorbed was excreted unchanged as α-pinene, β-pinene, and 3-carene in expired air; up to 4% of the total inhaled α-pinene was excreted in the urine as *cis*- and *trans*-verbenol (Filipsson, 1996).

The rate of metabolism of turpentine was increased by repeated or chronic exposure (Sperling and Ewenike, 1972; cited by Savolainen and Pfäffli, 1978). In male rats exposed to 300 ppm (1670 mg/m^3) turpentine in air for six hours per day, five days per week for four to eight weeks, hepatic microsomal enzyme activity was effectively induced (Jarvisalo and Vainio, 1980). Elevated liver microsomal epoxide hydrase and uridine diphosphoglucuronosyltransferase activity was present throughout the exposure period.

9.1.3 Acute Exposure

For the purpose of this report, acute exposures are defined as single or repeated exposures that occur in a single 24-hour period of time. Acute toxicity values in animals for turpentine are presented in **Table 10**. The details of acute animal studies discussed in this section are presented in **Table 11**.

As in humans, turpentine acts as a CNS depressant, with symptoms progressing from lethargy, prostration, and convulsions to death in animals (Bystrom, 2000). Acute toxicity in animals included irritation of the skin, eyes, nose, and mucous membranes. Signs and symptoms of acute toxicity are CNS depression and increased respiration rate with a decrease in tidal volume. Major systemic effects include kidney and bladder injury, and are thought to be attributable to induction of an acute inflammatory response (Key et al., 1977; cited by Baxter, 2001).

In mice exposed by inhalation to turpentine and its various constituents, the RD_{50} (the concentration that causes a 50% decrease in respiratory frequency) of turpentine (1173 ppm, 6536 mg/m^3) was the same order of magnitude as those of *d*-α-pinene (1053 ppm; 5867 mg/m^3), *d*-β-pinene (1279 ppm; 7126 mg/m^3), and *d*-Δ^3-carene (1345 ppm; 7494 mg/m^3) (Kasanen et al.,

Table 10. Acute Toxicity Values for Turpentine in Animals

Route	Species (strain and sex)	LD_{50}/LC_{50}	Reference(s)
Dermal	Rabbit (strain and sex n.p.)	LD_{Lo} = 5010 mg/kg (36.78 mmol/kg)	RTECS (2001)
Inh	Mouse (strain and sex n.p.)	LC_{50} = 29,000 mg/m^3 (5204.8 ppm)	Baxter (2001)
	Mouse (strain and sex n.p)	LC_{50} = 1615 ppm (8998 mg/m^3)	Domanski (1989; cited by Kasanen et al., 1999)
	Rat (strain and sex n.p.)	LC_{50} = 12,000 mg/m^3 (2153.7 ppm)	RTECS (2001)
	Rat (strain and sex n.p.)	LC_{50} = 3590 ppm (20,000 mg/m^3) (1 h exposure); 2150 ppm (11,980 mg/m^3) (6 h exposure)	Sperling et al. (1969; cited by ACGIH, 1991)
	Rat (strain and sex n.p).	LC_{50} = 20,000 mg/m^3 (3590 ppm) (1 h exposure)	Smyth and Smyth (1928; cited by Baxter, 2001)
	Rat (strain and sex n.p.)	LC_{50} = 12,000 mg/m^3 (2153.7 ppm) (6 h exposure)	Smyth and Smyth (1928; cited by Baxter, 2001)
	Rat and guinea pigs (strain and sex n.p.)	LC_{50} = 2423 ppm (13,500 mg/m^3) (6 h exposure)	Domanski (1989; cited by Kasanen et al., 1999)
	Guinea pig (strain and sex n.p.)	LC_{Lo} = 16,000 mg/m^3 (2871.6 ppm) (1 h exposure)	RTECS (2001)
	Cat (breed and sex n.p.)	LC_{80} = 2871 - 4307 ppm (15,997 – 23,998 mg/m^3) (40 min-1.5 h)	Sandmeyer (1981; cited by Kasanen et al., 1999)
i.v.	Mouse (strain and sex n.p.)	LD_{50} = 1.180 µmg/kg (8.662 µmol/kg)	RTECS (2001)
Oral	Rat (strain and sex n.p.)	LD_{50} = 5760 mg/kg (42.28 mmol/kg)	RTECS (2001)

Abbreviations: inh = inhalation; i.v. = Intravenous; $LC(D)_{\chi}$ = concentration (dose) lethal to χ% of test animals; $TC(D)_{Lo}$ = lowest concentration (dose) tested resulting in adverse effects; n.p. = not provided.

1999; Kasanen et al., 1998, cited by Kasanen et al., 1999). The effect on breathing was most likely due to sedation or anesthesia since no histological observations were reported in the exposed animals.

Craig and Franklin (1977) investigated the potential of turpentine to induce hyperplasia in a hamster cheek pouch model. In a time-course evaluation, the authors demonstrated an increase in epithelial thickness within 48 hours of a single cheek painting (six strokes, 50% turpentine in liquid paraffin).

The minimum concentration of turpentine in acetone that elicited a moderate degree of skin irritation free from ulceration was 30% (Berenblum, 1935). Older preparations contain higher concentrations of 3-carene, a constituent thought to be responsible for dermatologic toxicity (eczema) (Gosselin, et al., 1984; cited by ACGIH, 1991).

Table 11. Acute Exposure to Turpentine

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, OF1, M, 4-5 wk, total animals used = 100: 4 animals/repetition: turpentine - 9 repetitions Δ^3-carene - 14 repetitions control - 2 repetitions (room air)	Commercial sulfate turpentine (Oulu 1) Kiilto Ltd., Tampere, Finland, purity n.p.) and Δ^3-carene (Sigma Aldrich, purity n.p.)	Single 30 min inhalation of turpentine, Δ^3-carene, and room air for 30 min. Approximate exposure concentrations extrapolated from Fig. 1 were 100, 200, 350, 700, and 1000 ppm (557, 1110, 1950, 3900, 5572 mg/m^3)	The RD_{50} of turpentine (1173 ppm; 6535 mg/m^3) was the same order of magnitude as those of *d*-α-pinene (1053 ppm), *d*-β-pinene (1279 ppm), and *d*-Δ^3-carene (1345 ppm). No histological changes were seen in the lungs of exposed mice. The increase in the time of pause after expiration in mice that inhaled turpentine or 3-carene was most likely due to sedation or anesthesia and not to pulmonary irritation.	Kasanen et al. (1999)
Hamster, Syrian, M, 4-6 wks, 4 animals (part of larger subchronic study)	Turpentine, 50% v/v liquid paraffin (source, purity n.p.)	Dermal (painted cheek pouch, 6 strokes of camel hair brush); sacrificed 24 h (2 animals) and 48 h (2 animals)	Increased epithelial thickness observed within 48 h after single painting; inflammatory changes were noted for both epithelium and connective tissues.	Craig and Franklin (1977)
Cat, species, strain, sex, age, and number of animals n.p.	Turpentine (source and purity n.p.)	Inh: 4100 – 4300 mg/m3 (735.9 – 771.7 ppm) (further details n.p.) for 3.5-4 h Inh: 6000 mg/m3 (1077 ppm) for 3 h Inh: 8000 mg/m3 (1436 ppm) for 1-1.5 h Inh: 16000 – 24000 mg/m3 (2871.6–4307.4 ppm) (further details n.p.) for 1.5 h	Lethargy, incoordination, nausea Prostration with recovery within 20 min Incoordination Lethal for 4/5 cats.	Gosselin et al. (1984; cited by Baxter, 2001)
Cat, species, strain, sex, age, and number of animals n.p.	Turpentine (source and purity n.p.)	Inh: 540-720 ppm (3010 – 4010 mg/m^3) (further details n.p.), a few h Inh: 1440 ppm (8020 mg/m^3), 30-180 min	Eye and mucous membrane irritations; mild convulsions. 30–60 min exposures resulted in disturbances in equilibrium and tonic convulsions. Animals developed paralysis within 150-180 min.	Patty (1963, cited by HSDB, 2000)

Abbreviations: h = hour(s); inh = inhalation; M = male; min = minutes; ppm = parts per million; RD_{50} = concentration that causes a 50% decrease in respiratory frequency; wk = week(s); n.p. = not provided

9.1.4 Short-term and Subchronic Exposure

Short-term and subchronic exposure studies are defined as exposures of durations greater than 24 hours but less than 30 days and between 30 and 90 days, respectively. Details for studies that fall into these exposure categories are presented in **Table 12**. Often, turpentine is used simply as a positive control or an agent to induce a specific physiological response in an animal model for further study (Chapman, 1941; Craig and Franklin, 1977; Zarrabi et al., 1977; Raja et al., 1990). Other citations focused on the effects of turpentine itself (Smyth and Smyth, 1928; McIntosh et al., 1975; Savolainen and Pfäffli, 1978). One study (Greenwel and Rojkind, 1997) looked for a potential interaction between turpentine and carbon tetrachloride.

As a part of a study to determine safe exposure levels for a large number of solvents, inhalation toxicity of steam-distilled turpentine vapors (715 ppm; 3984 mg/m^3) was evaluated in guinea pigs exposed for four hours a day for 45 or 58 days (Smyth and Smyth, 1928). Although no significant hematological changes attributable to turpentine were detected, slight changes in the livers and moderate scattered tubular degeneration in the kidneys were observed.

In an early etiology study investigating Bright's disease, Chapman (1941) exposed rats to turpentine vapors (estimated at 5000 to 10,000 mg/m^3; 897.4 to 1794.8 ppm) for 30 minutes at a time until they succumbed to the effects. A total of 36 rats were exposed to turpentine vapors from 6.5 hours to 293 hours over a time frame ranging from two days to 14 months. Kidney sections from both exposed and control animals indicated no histological pattern that could be interpreted as being a form of nephritis or chronic Bright's disease. Most rats, however, had foci of pneumonitis and many had extensive lung abscesses, which were the likely cause of death.

In a study to determine the uptake α-pinene in rats, inhalation of turpentine at 300 ppm (1670 mg/m^3) over eight weeks resulted in an accumulation of α-pinene in the perinephric fat. Brain concentrations of α-pinene did not exceed one-tenth the concentration found in the fat. There was an initial decrease in brain RNA, followed by recovery. It was concluded that at modest exposures (300 ppm or 1/7 LC_{50} for a six hour exposure), there was clearly an acute effect on brain RNA; however, the effects observed were similar to those found with other solvents (Savolainen and Pfäffli, 1978). The short-term effects returned to control levels by the end of the experiment (eight weeks).

In a preliminary test for further initiation/promotion investigations, Roe and Field (1965) studied the effects of several essential oils, based on the premise that the irritant effects of these oils could lead to cancer induction as either co-carcinogens or tumor-promoting agents. Turpentine applied twice at weekly intervals resulted in moderate to marked epidermal hyperplasia which, with some of the chemicals tested, progressed to necrosis with ulceration, weeping, and crusting (no more specific information provided).

Raja et al. (1990) investigated the effects of inflammatory reactions on the uptake and transfer phase of intestinal iron absorption. Normocytic anemia (small, significant reductions in red cell

Table 12. Short-term and Subchronic Exposure to Turpentine

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, '101' inbred strain, 8 to 10 wk, sex and numbers n.p.	Turpentine (source and purity n.p.)	Turpentine applied dermally 7 d apart, to the back, from which the hair was removed. A biopsy of the dorsal skin was made 3 d after each application.	Moderate or marked epidermal hyperplasia was observed. In some cases, areas of necrosis with ulceration, weeping and crusting were seen.	Roe and Field (1965)
Mice, strain n.p., M, 6-7 wk-old, 7 turpentine-treated, 5 saline-treated controls	Turpentine oil (source and purity n.p.)	Turpentine (0.1 mL [0.09g; 0.6 mmol]) was injected s.c. into the intrascapular fat pad, 1x/wk for 6 wk.	Chronic turpentine treatment resulted in a mild normocytic anemia evident as a significant reduction in RBC mass, hematocrit and hemoglobin. However, no distinct changes were seen either in mucosal retention or mucosal transfer rates of iron in the turpentine treated mice.	Raja et al. (1990)
Rats, Wistar, M, adult, 40 treated, 40 control animals.	Commercial turpentine (95% α-pinene, purity n.p.)	Inh at 300 ppm (1670 mg/m^3), 6 h/d, 5 d/wk, up to 8 wk	5 rats were killed 1, 4, 5, 6, 7, and 8 wk after treatment start; 10 rats were killed 2 wk after treatment start. No behavioral abnormalities were observed in treated rats. Exposures resulted in the accumulation of α-pinene in fat; 1 and 2 wk exposures resulted in a reduction of RNA content in the brain, similar to the effects of other solvents.	Savolainen and Pfäffli (1978)
Rats, strain, sex and age n.p., 36 treated	Turpentine (source and purity n.p.)	Inh exposure (estimated between 5000 – 10,000 mg/m^3; 897.4-1794.8 ppm estimated concentration). Animals treated from 6.5-293 h, over a range of 2 d to 14 mo, until the animals died. Two animals were maintained for 60 to 210 d beyond the last exposure. This paper mentioned additional rats injected with turpentine i.v. or s.c. It was noted that these routes were problematic and few further comments were provided.	Inhalation: No gross pathological changes were observed in kidneys. Toxic signs included weakness and ataxia, bloody nasal discharge, paraplegia and eventually coma and death. Many rats showed some foci of pneumonitis and many had extensive lung abscesses that were the probable cause of death. i.v. injection: venous thrombosis, and on two occasions, death followed an injection of 0.5 mL of turpentine. s.c. injection: extensive abscesses leading to secondary infection and emaciation of the animals.	Chapman (1941)
Rats, Wistar, M, 350-500 g; 37 animals (part of larger experiment)	Steam-distilled turpentine (source and purity n.p.)	Animals (20) received i.m. injections in each hind leg 2x/wk for 5 wk; observation period unclear from paper. The control group had 17 animals.	Treated rats developed necrotic i.m. abscesses 1-2 cm in diameter, and experienced mild anemia. Blood volumes (as % body weight) in rats with abscesses were significantly below normal.	Zarrabi et al. (1977)

Table 12. Short-term and Subchronic Exposure to Turpentine (Continued)

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Rats, Sprague-Dawley, M, 80-100 g, 30 total animals (6 animals in the control group and 8 each in three treatment groups)	Turpentine (EM Science, Cherry Hill, NJ, purity n.p.)	I.d. injection of 100% turpentine (0.15 mL [0.13g; 0.95 mmol]) 1x/wk for 3 weeks. Animals were sacrificed 72 hours after last turpentine injection.	Histological examination of hepatic tissue revealed no gross changes in turpentine-treated rats. The concentration of liver collagen was also not altered. This is part of a larger study on interactions.	Greenwel and Rojkind (1997)
Guinea pigs, strain, sex, and age n.p., 4 animals exposed (part of a larger study)	Steam-distilled wood turpentine (source and purity n.p,)	Inh of 715 ppm (3980 mg/m^3). Animals were exposed daily (4h/d) for the first two wk; animals then were exposed 4h/d, 6d/wk for up to a total of 58 exposures.	One animal died after 12 treatments; it was replaced. The replacement animal was treated a total of 45 times; the two remaining animals were treated a total of 58 times. There were no abnormal blood changes. Slight changes in the livers and moderate scattered tubular degeneration in the kidneys were observed. The concentration of wood turpentine tested was considered to be relatively safe.	Smyth and Smyth (1928)

Abbreviations: b.w. = body weight; d = day(s); F = female; h = hour(s); inh = inhalation; i.d. = intradermal(ly); i.m. = intramuscular(ly); i.v. = intravenous(ly); M = male; min = minute(s); mo = month(s); n.p. = not provided; RBC = red blood cells; RNA = ribonucleic acid; s.c. = subcutaneous(ly); wk = week(s)(ly);

mass, hematocrit, and hemoglobin without changes in the mean corpuscular volume) was induced in mice by subcutaneous (s.c.) injections of turpentine into the intrascapular fat pad once a week for six weeks.

Similarly, Zarrabi et al. (1977) induced anemia in rats by intramuscular injections in each hind leg twice a week for five weeks in an attempt to evaluate the role of defective iron reutilization in the pathogenesis of chronic disease-associated anemia. Treated rats developed mild anemia and abscesses at the site of injections. Blood volumes were reduced relative to body weight, especially in animals with abscesses.

Turpentine injected intradermally (0.15 mL, 0.13g; 0.95 mmol) once a week for three weeks did not result in adverse pathological findings or altered collagen levels in livers of male Sprague-Dawley rats (Greenwel and Rojkind, 1997).

9.1.5 Chronic Exposure

No chronic toxicity studies for turpentine were identified.

9.1.6 Synergistic/Antagonistic Effects

Turpentine injected intradermally in male Sprague-Dawley rats contributed to liver toxicity induced by carbon tetrachloride (CCl_4 in mineral oil, i.p.) (Greenwel and Rojkind, 1997). Turpentine alone failed to produce any overt liver pathology. In combination with CCl_4, however, turpentine resulted in more fibrosis and less steatosis than the CCl_4 treatment alone. The steatosis in the combined treatment group was diffuse, though it affected predominantly zone 3 of the hepatic acinus. The combined-treatment animals also expressed more liver collagen than the CCl_4 treated group. The collagen fibers were thicker and formation of septa occurred more frequently in the CCl_4 plus turpentine than in the CCl_4 treated animals. Enhancement of liver fibrosis in rats treated with both CCl_4 and turpentine is not likely to be due to turpentine-induced liver toxicity, but rather to the induction of immune acute phase proteins such as fibrinogen, α_2-macroglobulin, and α_1 acidic glycoprotein, which enhance lipid peroxidation by CCl_4.

Turpentine administered orally (1.8 mg/kg; 0.013 mmol/kg) to rats for three days reduced parathione toxicity. The proposed mechanism for this protection was through induction of microsomal enzymes (Walker and Colwell, 1976; Norton, 1975; both cited by Baxter, 2001).

Other interactions associated with turpentine treatment involve protection against inflammatory responses. Guinea pigs were protected from hypersensitivity reactions to 6-mercaptopurine by treatments with turpentine (Homburger and Boger, 1968; cited in Baxter, 2001). Similarly, rats pretreated with turpentine demonstrated a reduction in the development of carrageenan-induced edema (Deflandre et al., 1973; cited by Irwin et al., 1997).

9.2 Reproductive and Teratological Effects

The details of reproductive toxicity or teratogenicity studies are provided in **Table 13.**

In rats, *in utero* exposures to turpentine during late gestation led to CNS depression at birth (Garcia-Estrada et al., 1988). In addition, there was a 59% mortality rate among the exposed litters. The dams were exposed to turpentine vapors twice daily for ten minutes, on gestation days 17 to 21. Experimental animals demonstrated signs of incoordination, salivation, and polypnea within the first five minutes of each exposure. *In utero* exposed pups exhibited dyspnea and severe CNS depression at birth, but no gross histopathological abnormalities were observed in the brains of the treated animals

9.3 Carcinogenicity

No scientifically adequate studies on turpentine were located.

9.4 Initiation/Promotion Studies

Details of the initiation/promotion studies are presented in **Table 14**.

An increased multiplicity of site-of-application tumors per mouse was observed in mice initiated with dimethylbenzanthracene (DMBA) followed by treatment with turpentine (Boutwell and Bosch, 1959; Roe and Pierce, 1960; cited by Roe and Field, 1965; Frei and Stephens, 1968). α-Pinene was reported to have the same promotional activity as turpentine (Roe and Field, 1965).

In an ear skin-painting study of a variety of agents, it was found that turpentine was a mild promoter after initiation with DMBA (Frei and Stephens, 1968). Animals had their ears painted with DMBA, followed one week later with one of several promoting agents. Results were assessed 20 weeks later. One of 18 Swiss mice painted with 50% turpentine in mineral oil exhibited one tumor after 20 weeks of treatment with an average of 0.06 tumors/survivor. This incidence was not significantly increased over the zero incidence of tumors among the control mice. In the DMBA initiation study followed by turpentine promotion, 71% of the survivors had tumors, with an average of 2.2 tumors/survivor. Relative to the promoting powers of croton oil (88% survivors had tumors with an average of 10.7 tumors per survivor), turpentine was considered a mild promoter.

Donawho et al. (1994) examined the impact of turpentine-induced inflammation on the growth of the transplantable melanoma K1735. Ultraviolet radiation (UVR) acts as a tumor initiator, tumor promoter and co-carcinogen, possibly through modulations of the immune system. When the melanoma K1735 was injected into the UV-irradiated ears of syngenic C3H/HeN(MTV-) mice, there was both an increase in the incidence of tumor formation and a reduced latency compared with the non-irradiated, tumor treated controls. The ears of mice were treated with turpentine (10 μL] (one to six treatments, twice a week, over a three week period) to induce a similar degree of inflammation as UVR. Although turpentine treatment resulted in an inflammatory response similar in time course, magnitude and histological appearance to that induced by UVR, the turpentine treatment failed to promote the incidence of melanomas.

Table 13. Reproductive Toxicity and Teratology of Turpentine

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Rats, Sprague-Dawley, F, adult, 5 treated and 3 control rats	Turpentine (source and purity n.p.)	Pregnant rats were exposed for 10 min, 2x daily (10 min each exposure) to turpentine vapors by inh (concentration n.p.) from GD 17-21.	Within 5 min of exposure, experimental animals demonstrated signs of incoordination, salivation and polypnea. A 59% mortality rate was observed in progeny of turpentine-exposed pregnant rats. Pups derived from turpentine-treated dams had a decreased birth weight relative to control pups. Body weight at birth was a good predictor of survival; pups that died after birth were generally smaller than the surviving pups. No progeny from control rats died. Exposed pups exhibited dyspnea and severe CNS depression symptoms at birth, but no gross histopathological observations in the brains.	Garcia-Estrada et al. (1988)

Abbreviations: CNS = Central Nervous System; F = Female; GD = gestation day; inh = inhalation; min = minutes; n.p. = not provided

Table 14. Initiation/Promotion Studies of Turpentine

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, Swiss, M, 6- to10-wk-old, 60	Turpentine (commercial formulation from Record Chem. Co. of Montreal Canada, purity n.p.)	Mouse ears were painted with once with 1-5% DMBA; beginning 1 wk after DMBA treatment, ears were painted with 100% turpentine 2x/wk for 20 wk. 30 mice initiated with DMBA only.	2 groups of 30 mice were treated with turpentine. Group A received the initiator DMBA prior to ear painting with turpentine; Group B received the turpentine treatment only. Any lesion that was >1 mm in diameter and was seen for at least three consecutive weeks was identified as a tumor. In Group A, 21 mice survived to the end of the experiment; of those survivors 71% bore tumors. The average number of tumors per survivor was 2.2. In Group B, 18 mice survived to the end of the experiment and only 1 bore one tumor. Turpentine acted as a mild tumor promoter under this experimental condition. In the DMBA only treatment group, 1/23 survivors (4%) had one tumor (not significantly different from control group), or 0.04 tumors/survivor.	Frei and Stephens (1968)
Mice, Sutter, F, 2- to 3-mo-old, 20 animals in the turpentine-treated group	Turpentine (Distillation Products Industries, Inc. Rochester, NY, purity n.p.)	A single application of 0.3% DMBA in benzene (amount n.p.) to mouse backs, then 2 drops (approx. 50 µL) of 100% turpentine applied to site-of-application 6x/wk for 20 wk.	DMBA was used as the initiator and turpentine was tested for its ability to act as a promoter. Of the surviving 18 DMBA-initiated, turpentine-promoted mice at the end of the experiment, 3 (17%) exhibited papillomas. The average multiplicity of papillomas per survivor was 0.28. No malignant tumors were induced. DMBA initiation without subsequent promotion resulted in 18/20 survivors at 20 weeks. There were no observable papillomas in the control group.	Boutwell and Bosch (1959)
Mice, '101' strain (inbred) and stock albino (random-bred), 8 wk-old, sex and number n.p.	Turpentine (source and purity n.p.)	Mice initiated with 300 µg (turpentine group) or 150 µg (α-pinene group) DMBA in 2.0 mL (0.22g, 1.6 mmol) acetone followed three wks later by either 0.25 mL undiluted turpentine oil or 40% α-pinene in acetone once a wk. Total length of study: 33 wk.	Weak tumor promotion was observed in turpentine- and α-pinene-treated mice. 8 of 19 surviving mice dosed with turpentine had papillomas (0.54 papillomas/survivor) and 3 of 15 α-pinene-dosed mice had papillomas (0.27 papillomas/survivor). In DMBA treatment only, one of 16 survivors had a single papilloma; this single papilloma occurred outside the exposed area.	Roe and Peirce (1960, cited in Roe and Field, 1965)

Table 14. Initiation/Promotion Studies of Turpentine (Continued)

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, SPF [C3H/HeN (MTV-)], F, 8- to 12-wk-old, number n.p.	Parks pure gum turpentine (source and purity n.p.)	Ears of mice were painted with turpentine [10 µL] 1-6 times over a 3-wk period, then K1735 melanoma cells were injected into the ears 10 d after the last turpentine application; other groups of mice were treated with UVB radiation 20 min 2x/wk for 1 to 5 wk or TPA (0.0005% in acetone) with exposure the same as turpentine	Mice were initially treated twice/wk with turpentine, but the escalating inflammatory response, along with necrosis and severe tissue damage made it necessary to change the treatment to one application per wk. The swelling curves for TPA, UVB, and turpentine were similar, with turpentine swelling peaking around 0.25 mm at 9 d and TPA- and UVB-treated mice around 0.16 mm at 9 d. Microscopic examination of the ears showed that at the time of tumor cell injection there were similar histopathologic changes in the turpentine-treated and UV-irradiated mice; however, the TPA-treated group showed more extensive epidermal hyperplasia and mononuclear cell infiltrate in the dermis than the turpentine-treated and UVB-irradiated groups. Tumor growth was enhanced in mice irradiated with UVB, when compared to untreated control mice. The growth of melanomas in the TPA- and turpentine-treated groups was less than growth observed in untreated control mice.	Donawho et al. (1994)
Rabbits, New Zealand white, M and F, age and number n.p.	Turpentine and acetone (Fisher Scientific, PA.; purity n.p.)	One half of the backs of rabbits were painted with a turpentine and acetone (50/50) mixture 4 times every other day; the other half was painted with PBS. Rabbits then received an inoculation of plasmid containing CRPV DNA	There was a 4-fold increase in skin thickness in turpentine- and acetone-treated skin when compared to skin of rabbits treated with PBS. The epidermal thickness observed in turpentine- and acetone-treated rabbits was similar to that seen after treatment with 250 µg/ml of phorbol-12-myristate-13-acetate. Skin treated with turpentine and acetone was much more responsive to papilloma growth than skin treated with saline. Some rabbits were also mechanically treated to induce either light or heavy scarification. Rabbits that received heavy scarification only exhibited 45% (9/20) papillomas. Rabbits that received light scarification yielded 35% (8/20) papillomas. Rabbits that received both heavy scarification and turpentine/acetone treatment produced 95% (19/20) papillomas.	Kreider et al. (1995)

Abbreviations: CRPV = cottontail rabbit papilloma virus; d = day(s); DMBA = 7,12-dimethylbenz(a)anthracene; DNA = deoxyribonucleic acid; F = female; M = male; mo = month(s); n.p. = not provided; PBS = phosphate buffered saline; TPA = 12-O-tetradecanoylphorbol-13-acetate; UVR = ultraviolet radiation; wk = week(s);

It had been previously demonstrated that turpentine (50% in acetone) pretreatment could increase the susceptibility of rabbit skin to virion-induced papillomas (Friedewald, 1942; cited by Kreider et al. 1995). Kreider et al. (1995) extended this concept by systematically investigating the efficiency of several hyperplasia-inducing methods in the development of papillomas. New Zealand white rabbits had one half of their backs treated with turpentine (50% in acetone) and the other half treated with phosphate buffered saline (PBS) then inoculated with cottontail rabbit papillomavirus (CRPV). The turpentine side demonstrated significantly enhanced papilloma growth relative to the PBS treated side (Kreider et al., 1995).

9.5 Anticarcinogenicity

The details of papers pertaining to the anticarcinogenicity of turpentine are presented in **Table 15**.

Berenblum (1935) discovered that in mustard gas and related compounds, tumor inhibition correlated directly with the degree of skin irritation. Therefore, a number of known skin irritants, including turpentine, were tested for their ability to inhibit tumor formation. Although capable of causing skin irritation similar in nature to that induced by mustard gas, the combined treatment of tar and 50% turpentine in acetone, failed to have any observable effect on the development and growth of tar-induced tumors (Berenblum, 1935).

Caldwell and Archibald (1987) injected C57 black mice subcutaneous with turpentine during EL4 lymphoma cell growth to determine if the induction of a hypoferremic response would have an impact on tumor growth. Reducing the amount of available iron to the rapidly growing tumor cells had no effect on growth.

9.6 Genotoxicity

No genotoxicity studies were found for turpentine.

9.7 Cogenotoxicity

No cogenotoxicity studies were found for turpentine.

9.8 Antigenotoxicity

No antigenotoxicity studies were found for turpentine.

9.9 Immunotoxicity

The details of the following study are presented in **Table 16**.

Electron microscopic studies of macrophages of granulomas in rats intramuscularly exposed to turpentine revealed many inclusions including lamellar bodies (Fuchs, 1966). It was concluded that quantitative and qualitative differences exist between turpentine-induced inflammation and other models of inflammation. The turpentine oil was so toxic that it not only damaged the preexisting structures, but also the newly forming granulation structures.

Human sensitization studies were presented in **Section 9.1.1.2**.

Table 15. Anticarcinogenicity Studies of Turpentine

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, C57 BL/6, M, 6- to 8- wk-old, 44 per dose group	Turpentine (source and purity n.p.)	Mice were injected s.c. with turpentine (0.05 mL [0.04g; 0.3 mmol]) in two sites on the back; 1 h later mice injected i.p. with 10,000 tumor cells (EL4 lymphoma [ATCC TIB 39])	A hypoferremic response was induced in mice by the injection of turpentine. The resulting hypoferremia, which lasted about 48 and 72h, did not inhibit the growth of tumor cells.	Caldwell and Archibald (1987)
Mice, strain, age and sex n.p., 50 per dose group	Turpentine (source and purity n.p.)	Animals painted 1x/wk with tar and 50% turpentine in acetone, on separate days, with camel hairbrush for up to 20 wk	Turpentine treatments resulted in thickening of skin, some hair loss, but no ulceration (similar to that of mustard gas). A control group received the tar painting only. There was inhibition of tumor growth between the tar-treated group and the group treated with both tar and turpentine. There were 36 survivors in the turpentine and tar treated group after 15 wk; 50% of the survivors to developed tumors by wk 16.	Berenblum (1935)

Abbreviations: F = female; h = hour(s); i.p. = intraperitoneal(ly); M = male; n.p. = not provided; s.c. = subcutaneous(ly); wk = week(s);

Table 16. Immunotoxicity of Turpentine

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Rats, strain, sex, age, and number n.p.	Turpentine (source and purity n.p.)	i.m. injection, dose, duration, and observation period n.p.	Electron microscopic studies of macrophages of granulomas in rats exposed i.m. to turpentine revealed many inclusions, including lamellar bodies. Larger, loosely layered membranous bodies were more rare. It was concluded that quantitative and qualitative differences exist between turpentine-induced inflammation and other models of inflammation. The turpentine oil was so toxic that it not only damaged the preexisting structures, but also the newly forming granulation structures.	Fuchs (1966); article in German w/English abstr.

Abbreviations: i.m. = intramuscular; n.p. = not provided

9.10 Other Data

Other data found for turpentine are described in **Table 17**; the studies identified investigated turpentine-induced hyperplasia and inflammation.

Turpentine treatment induces an acute response resulting in the release of many inflammatory mediators from the liver (Baxter, 2001). In a study of turpentine-induced inflammation (species and route not reported), a 1.6- to 2.3-fold increase in liver homogenate sialyl-, galactosyl-, and *N*-acetylglucosaminyltransferase was noted. The rise and fall of these activities corresponded with the rise and fall of serum haptoglobin (Lombart et al., 1980; cited by HSDB, 2000). Hong and Shin (1977; cited by HSDB, 2000) noted a similar effect in mice and rabbits and suggested that turpentine stimulated the synthesis and massive release of the haptoglobin from the liver into the circulation. Turpentine treatment also resulted in an increase in ceruloplasmin activity along with the release of other acute phase proteins. This release was strongly influenced by dietary copper content. Ceruloplasmin is thought to inhibit inflammatory injury, possibly by acting as an antioxidant (DiSilvestro, 1989).

Turpentine challenge of normal and busulfan-treated rabbits resulted in a sharp increase in circulating plasma fibrinogen, with a more profound effect observed in the normal rabbits (Rapaport and Zivelin, 1976; cited by HSDB, 2000).

At low doses, turpentine protects against the response induced by other agents. Deflandre et al. (1973; cited in Irwin, 1997) demonstrated a reduction in the development of carrageenan-induced edema by pretreating with turpentine.

One of the early theories regarding the two-stage cancer model suggested a requirement for a marked and sustained hyperplasia for the induction of tumor formation (Roe and Field, 1965). Based on turpentine's known skin irritancy properties, several studies have investigated turpentine-induced hyperplasia as a model system. In 1977, Craig and Franklin studied the potential for turpentine to produce epithelial hyperplasia in the hamster cheek pouch. The authors described several different dosing regimes in evaluating turpentine-induced hyperplasia. Cheek pouches were painted three times a week for up to 16 weeks with 50% turpentine in liquid paraffin. An increase in the epithelial thickness was observed within 48 hours after a single painting. Maximal thickening was observed at nine weeks, with many areas of the epithelium demonstrating a three to four fold increase in thickness. The inflammatory changes encompassed both the epithelium and connective tissue. In some cases, cheek painting was discontinued after nine weeks and animals were followed for up to one year. At that time the treated cheeks were indistinguishable from control cheeks. Based on the results of these studies, the authors concluded that this represented a good animal model for reversible, chemically-induced benign epithelial hyperplasia with hyperkeratosis and predicted that the model would be useful for the biological and physiological evaluation of hyperplastic epithelium, which has no malignant potential.

Table 17. Studies of Turpentine-induced Hyperplasia and Inflammation

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, Swiss, M, 6- to 10-wk-old, multiple dose groups, 10 animals per group	Turpentine (Record Chemical Co., Inc., Montreal, purity n.p.) and *d*-limonene (Eastman Organic Chemicals, Rochester, N.Y., purity n.p.)	Ears were painted with either 100% turpentine or 20% *d*-limonene in mineral oil 2x/wk for up to 50 d.	A set of 10 ears was removed on days 2, 5, 10, 20, and 50. Ears were examined for increased epidermal cell number, variations in epidermal thickness, and the presence of cellular inflammatory exudate. Both turpentine and *d*-limonene induced hyperplasia and cellular inflammatory exudate. Treatment with 20% limonene or 100% turpentine produced a significant increase in epidermal thickness by 10 days; the thickness remained high, but not significant to the end of the observations period (50 d).	Frei and Stephens (1968)
Mice, Swiss, M, 6- to 10-wk-old, number n.p.	Turpentine (commercial formulation from Record Chem. Co. of Montreal Canada, purity n.p.)	Mouse ears were painted with one of several agents (including DMBA, croton oil, and turpentine) once and sampled 10 or 20 h later. Animals were injected i.p. with ^{3}H-thymidine 1 h prior to sampling (9 and 19 h post-treatment, respectively)	No significant increase in ^{3}H-thymidine uptake at either 10- or 20-h post turpentine-treatment relative to the mineral oil control treatment. Both DMBA and croton oil showed a positive response.	Frei and Stephens (1968)
Mice, ICR (Hanuschka), 10-15 animals/group, 4 treatment groups plus 2 untreated controls; adult; sex n.p.	Turpentine (source and purity n.p.)	Backs were painted 2x/wk with 0.5% turpentine in acetone; 3-MC (0.05 ml of 0.03% in acetone), or croton oil (0.05 ml of 1% in acetone); animals were sacrificed at 1, 3, 6, 12, 24, or 72 h (animals only received one treatment) or 1, 5, 8, 12 wk after the first treatment.	Amount of DNA/cell nucleus was measured at a single wavelength of 555 mμ. Histological examination showed considerable thickening of the epidermis. Hyperplasia induced by turpentine was similar in appearance as that induced by 3-MC, but occurred at a later date. DNA content/cell nucleus for the turpentine treated skin remained near diploid range (45 – 60% of cells). 3-MC-induced carcinomas had a significant increase (approaching triploid) in DNA/cell nucleus. Based on the number of animals used, only one or two animals were sacrificed at any one time point per dose group.	Inui and Takayama (1968)

Table 17. Studies of Turpentine-induced Hyperplasia and Inflammation (Continued)

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Rats, Sprague-Dawley, M, weanlings, 5 – 7 animals per group, total number of animals n.p.	Turpentine (source and purity n.p.)	One half of the animals received turpentine (0.1 ml [0.1g; 0.7 mmol], i.m.) 3 d prior to sacrifice.	Animals were fed diets (5 wk) with varying concentrations of copper (15 ppm, 6 ppm, 3 ppm, and 0.3 ppm). One half of the animals were injected with turpentine to see the combined impact of inflammation and copper status on the production of ceruloplasmin. Without inflammation, rats fed copper at 6 ppm (adequate copper balance) had approximately 50% of the ceruloplasmin activity in the 15 ppm copper group, as measured by oxidase activity toward *p*-phenylenediamine. Animals fed 6 ppm copper followed by induction of inflammation by turpentine responded with an increase in ceruloplasmin activity approaching the levels found in the 15 ppm copper treated animals. Ceruloplasmin activity was highly dependent on copper status.	DiSilvestro (1989)
Rats, Sprague-Dawley, 100-150 g b.w. (sex n.p.), 53 experimental animals plus 45 control animals, divided into 3 groups.	Turpentine (source and purity n.p.)	One group (53 rats) had one kidney wrapped in a turpentine-soaked silk pouch (volume n.p.). Surviving rats were sacrificed at 2, 5, and 8 wk after surgery. Control groups: 15 animals had one kidney wrapped in untreated silk; 15 animals were injected with small amount of turpentine i.m. (volume n.p. but approximately equivalent with the treatment group); third group untreated.	*In situ* exposure: the treated kidney showed a high incidence of proteinuria, diuresis and adverse kidney morphology, including perinephritis, acute tubular or cortical necrosis, and unilateral or bilateral glomerular fibrinogen deposition during the first 2 wk after treatment. After 2 wk, protein in the urine and urine volume began to normalize, but recurrent proteinuria, hypergammaglobulinemia, morphological alterations, and deposition of IgG and β1C on the glomerular basement membranes and mesangium of the contralateral kidney and the treated kidney continued. None of the control groups (including turpentine i.m.) showed any changes.	McIntosh et al. (1975)
Hamsters, Syrian, M, 4- to 6-wk-old; 5 subgroups, 3 animals each	Turpentine (source and purity n.p.)	Each cheek pouch was painted 3x/wk with 50% turpentine in liquid paraffin for 16 wk	Animals were killed 72 h after their last treatment at wk 2, 5, 9, 12, and 16. The maximal epithelial response occurred at about 9 wk (average thickness for keratin and epithelium was 92.34±19.4 vs. 41.08±19.4 μm in control animals). The painted mucosa exhibited few inflammatory changes and when present they amounted to scattered collections of chronic inflammatory cells in the sub-epithelial connective tissue. With continued painting, the keratin became thicker and the epithelium became thinner. There was no evidence of abnormal pathology in other organs.	Craig and Franklin (1977)

Table 17. Studies of Turpentine-induced Hyperplasia and Inflammation (Continued)

Species, Strain, Sex, Age, and Number of Animals	Chemical Form (Source and Purity)	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Hamsters, Syrian, M, 4-to 6-wk-old, 5 animals/group; 2 groups	Turpentine (source and purity n.p.)	Ears of each animal were painted 3x/wk for 9 wk. 5 animals from each group were observed for an additional 6 and 12 mo	Both groups exhibited a slight thickening of the epithelium, though non-significant compared to the control group.	Craig and Franklin (1977)
Hamsters, Golden Syrian, sex n.p., 4- to 6-wk-old, 2 groups of 16 animals each	Turpentine (source and purity n.p.)	Turpentine treated animals (n = 16): Right and left cheek pouches were painted (minimum of strokes with a No. 4 sable hair brush) with 50% turpentine v/v and mineral oil 3x/wk for 11 wk then applications were daily for 3 wk; DMBA treated animals (n = 16): Right pouch painted (as above) with 0.5% DMBA in mineral oil; left pouch painted with heavy mineral oil alone 3x/wk for 12 wk.	Failed to find significant hyperkeratosis or hyperplasia in hamsters treated with turpentine in mineral oil when compared to other cheek pouches treated with mineral oil or nothing at all. Some keratinization was noted with occasional focal areas, but the same observations were seen in animals treated with mineral oil only. DMBA hamsters had histologic lesions that were considered moderate dysplasia, severe dysplasia, carcinoma *in situ*, epidermoid carcinoma, or squamous papilloma.	Miller et al. (1988)
Hamsters, Bio 87.20, M, 4- to 6 wk-old, 20 per dose group	Spirits of turpentine (turpentine oil) (Stevens Chemicals, Christchurch, New Zealand; purity n.p.)	Both cheek pouches were treated with spirits of turpentine (50% v/v) in liquid paraffin (mineral oil) 3x/wk for either 2 or 4 wk; another group of mice (n = 25) received DMBA (0.5% w/v in liquid paraffin) in the same manner as described above.	Hamsters were divided into 5 dose groups. One group had cheek pouches removed 24 h after treatment for 2 wk. 4 other groups of 5 had cheek pouches removed 24 h, 6 wk, 12 wk, and 18 wk after painting for 4 wk. Histological comparisons were made with untreated control hamsters. Hyperplasia was produced by turpentine after 2 wk of application. An increase in epithelial thickness occurred after 4 wk of application. 12 wk after ceasing turpentine application, the epithelium had a normal histologic appearance. The neoplastic agent DMBA caused an increase (2- to 3-fold) in the concentration of low-molecular-weight cytokeratins and a decrease in the amount of high-molecular-weight cytokeratins. Through staining with Coomassie blue or electroblots reacted with polyclonal antibodies, it was found that the concentration of cytokeratins was the same in turpentine-treated cheek pouches as in those of controls.	Shearer et al. (1994)

Abbreviations: d = day(s); DMBA = 9,10-dimethyl-1,2-benzanthracene; F = female; h = hour(s); i.m. = intramuscular(ly); M = male; 3-MC = 3-methylcholanthrene; n.p. = not provided; wk = week(s)(ly

In a series of experiments, Frei and Stephens (1968) examined co-carcinogenicity and cell proliferating actions of a number of agents including turpentine and *d*-limonene in an attempt to identify changes that may be a part of the co-carcinogenic process. After treatment (two times per week) for up to 50 days, the ears were harvested at various time points and examined for several characteristics thought to be important in the initiation/promotion process, such as epidermal cell number, variations in epidermal thickness, the presence of cellular inflammatory process and hyperplasia. Turpentine (100%) and *d*-limonene (20% in mineral oil) both resulted in significant increases in epidermal thickness ten days after beginning treatment; epidermal thickness remained significantly high on day 50 for turpentine. Likewise, hyperplasia and cellular inflammatory exudate was significantly increased at day ten for both turpentine and *d*-limonene. Based on these results, turpentine was assessed in an initiation/promotion assay (see Section 9.4).

These authors also examined the uptake of tritiated thymidine in ears treated with hyperplasia-inducing agents, including turpentine. Tween 60, croton oil, and DMBA all resulted in an increase in thymidine uptake, indicative of DNA replication, after a lag of about 10 hours. Turpentine failed to increase thymidine uptake to any significant degree relative to the mineral oil-treated control ears. This work supported the theory that the second phase of the two-stage cancer model required a marked and sustained hyperplasia (Frei and Stephens, 1968).

Inui and Takayama (1968) investigated the differences between DNA content per cell nucleus in carcinomas and hyperplasic skin cells. Depilated back skin was treated with either 3-methylcholanthrene (3-MC), croton oil, or turpentine (0.05 ml of 0.5%), twice a week for up to12 weeks. The skin was harvested at various time points (1 hour to 12 weeks) after first treatment for histopathological examination and analysis of nuclear DNA content. All three compound induced hyperplasia, though at different time points, 3-MC being earliest. 3-MC resulted in the development of squamous carcinomas at 18 to 19 weeks; neither croton oil nor turpentine produced tumors. Of the three treatments, only 3-MC resulted in significant increases in DNA content in cell nuclei, approaching triploid values, and only in the carcinoma cells. The DNA content of turpentine-induced hyperplastic cells was not different from control cells.

In an attempt to study the discriminative value of toluidine blue in staining premalignant and malignant lesions compared to turpentine-induced hyperplastic lesions, Miller et al. (1988) utilized the turpentine and liquid petroleum (TLP) hamster cheek pouch model described by Craig and Franklin (1977) (see above). Under the experimental conditions described by Miller, however, the TLP model failed to produce either clinically or histologically evident hyperplastic/ hyperkeratotic lesions.

Turpentine oil was used as one of four hyperplastic agents used to distinguish changes in cytokeratin expression associated with reversible hyperplasia from the changes associated with carcinogenic hyperplastic processes as possible markers for early detection of malignant disease. Vitamin A palmitate, TPA, and ethylphenylpropiolate made up the rest of the panel of

hyperplastic agents. Treatment with the neoplastic agent DMBA resulted in changes in the concentrations of both the high and low molecular weight cytokeratins. Although there were transient increases in hamster cheek pouch epithelial thickness as a result of painting with turpentine oil, there were no differences in the immunohistochemical staining patterns of cytokeratins from cheeks of turpentine treated compared to control animals (Shearer et al., 1994).

McIntosh et al. (1975) investigated *in situ* kidney exposure to turpentine as a means to induce unilateral kidney disease. One kidney was exposed to turpentine by wrapping it in a piece of turpentine-soaked silk. The silk, in turn, was covered by a piece of plastic to ensure that only the single kidney was exposed. The treated kidney demonstrated adverse kidney morphology, including perinephritis, acute tubular or cortical necrosis, and unilateral or bilateral glomerular fibrinogen deposition during the first two weeks after treatment. Although urine and urine volume normalized after the first two weeks, there were recurrent episodes of proteinuria and hypergammaglobulinemia along with morphological alterations. Depositions of immunoglobulin G and β1C on the glomerular basement membranes and mesangium of both the treated and contralateral kidney occurred, suggesting systemic effects.

10.0 Structure-Activity Relationships

The acute toxicity of turpentine (see **Table 10**) is similar to the acute toxicity of its constituents (**Table 18**). It is thought that the *d*-enantiomers of the monoterpenes are more toxic than the *l*-enantiomers (Kasanen et al., 1999). A recent paper provides a review of the comparative toxicity of turpentine and its major constituents the pinenes, limonene, and 3-carene. The RD_{50} values reported for mice exposed to turpentine and its *d*-enantiomeric constituents were found to be similar (Kasanen et al., 1999; Kasanen et al., 1998, cited by Kasanen et al., 1999).

10.1 *α*-Pinene [CAS RN 81-56-8] and *β*-Pinene [CAS RN 127-91-3]

Literature searches of TOXLINE and PubMed databases using combinations of alpha pinene, pinene, terbenthene along with the appropriate CAS RN and skin, dermal, topical, neoplasia, neoplasms, neoplastic, tumor or tumour, subchronic, chronic or long term resulted in about 17 unique hits. Most of the results were associated with human responses to one or several of the monoterpenes. No chronic or carcinogenicity studies were identified with this search strategy. Although *α*-pinene was occasionally discussed by itself, *β*-pinene was always discussed in combination with other monoterpenes.

<u>Human Data</u>: Much of the language used for both *α*- and *β*-pinene was derived directly from excerpts about turpentine, based on the generalized observation that "Toxic effects [are] similar to turpentine" (Budavari, 1996). Furthermore, when secondary sources have cited the specific toxic effects from Budavari, they have cited those associated with the oleoresin [CAS RN 9005-90-7] rather than oil of turpentine [CAS RN 8006-64-2]. Toxicity associated with the oleoresin includes the development of benign tumors after chronic exposures (Budavari, 1996). Although the statement regarding the similarity of toxic effects is not made for *β*-pinene in Budavari, other sources have also associated the toxic effects described for the oleoresin with *β*-pinene.

Table 18. Acute Toxicity of Turpentine Constituents

Route	System	LC/LD_{χ}	Source
Inh	Mouse, rat, mouse, guinea pig (α-pinene from wood turpentine)	LC_{100} = 4666 ppm (26,000 mg/m^3) (5 h) (all species)	Domanski (1989); cited by Kasanen et al. (1999)
	Mouse, rat, mouse, guinea pig (α-pinene from sulfate turpentine)	LC_{100} = 5025 ppm (28,000 mg/m^3) (5 h) (all species)	Domanski (1989); cited by Kasanen et al. (1999)
	Mouse (α-pinene)	LC_{Lo} = 364 mg/m^3 (65.3 ppb)	NTP (2001)
	Mouse, rat, guinea pig (β-pinene from sulfate turpentine)	LC_{100} = 3517 ppm (19,596 mg/m^3) (5 h) (all species)	Domanski (1989); cited by Kasanen et al. (1999)
	Mouse [(+)-β-pinene]	RD_{50} = 1279 ppm (7126 mg/m^3) (30 min)	Kasanen (1989); cited by Kasanen (1999)
	Mouse [(-)-β-pinene]	RD_{50} = 4663 ppm (25,981 mg/m^3) (30 min)	Kasanen (1989); cited by Kasanen (1999)
	Rat (α-pinene)	LC_{Lo} = 625 mg/kg (4.59 mmol/kg)	NTP (2001)
	Guinea pig (α-pinene)	LC_{Lo} = 0.572 mg/m^3 (103 ppb)	NTP (2001)
p.o.	Mouse (*d*-limonene)	LD_{50} = 5.6 –6.6 g/kg	NTP (1990, cited by HSDB, 2002a)
	Rat (α-pinene)	LD_{50} = 2100 - 3700 mg/kg (14.52 – 27.16 mmol/kg)	Gscheidmeier and Fleig (1996)
	Rat (β-pinene)	LD_{50} = 4700 - 5000 mg/kg (34.50 – 36.70 mmol/kg)	Gscheidmeier and Fleig (1996)
	Rat (3-carene)	LD_{50} = 4800 mg/kg (35 mmol/kg)	Gscheidmeier and Fleig (1996)
	Rat (camphene; *d*,*l*-limonene)	LD_{50} > 5000 mg/kg (36.70 mmol/kg)	Gscheidmeier and Fleig (1996)
i.p.	Mouse (*d*-limonene)	LD_{50} = 1.3 g/kg	NTP (1990, cited by HSDB, 2002a)

Abbreviations: inh = inhalation; i.p. = intraperitoneal(ly); $LC(D)_{\chi}$ = the concentration (dose) that is lethal to χ% of test animals; $LC(D)_{Lo}$ = the lowest concentration (dose) that resulted in death of test animals; p.o. = per os; RD_{50} = the concentration that causes 50% decrease in respiratory frequency

Inhalation and dermal occupational exposures to the pinenes are likely to occur wherever they are produced or used. α-Pinene has been detected in the air of an electrical cable extrusion area (0 to 5μg/m^3) and at a telephone switching facility (0.39 to 0.44 μg/m^3). α-Pinene has been detected in the air samples collected from a wide variety of industrial facilities. β-Pinene was detected in the emission of a thermo-mechanical pulp production plant. In addition, garbage trucks emit β-pinene into the surrounding air, providing a potential exposure for workers picking up household waste. The general population may be exposed to these pinenes through the use of consumer products such as colognes, soaps, and fragrances (α- and β-pinene) and in foods (α-pinene, used as a flavoring component) (HSDB 2002b, 2002c).

Both α- and β-pinene are listed as irritants to the skin, eye, and mucous membranes (HSDB, 2002b, 2002c). Acute toxicity values for the two pinenes are very similar to the toxicity values provided for turpentine. The mean lethal dose of α-pinene is listed as between four and six oz (120 and 180 mL) turpentine. Fifteen mL (0.5 oz) was fatal to one child, while others have survived two and even three oz (60 or 90 mL) (Gosselin et al., 1984; cited by HSDB, 2002b). The probable oral human lethal dose of β-pinene ranged from 0.5 to 5 g/kg, or between 1 oz and 1 pint (30 and 470 mL) for a 70 kg (150 lb) individual (Gosselin et al., 1976; cited by HSDB, 2002c).

Studies in Finland and Sweden have focused on exposure at sawmill plants and joinery shops. Typically the exposures are classified as monoterpenes, specifically identifying α- and β-pinene and carene on occasion. Personal exposures in joinery shops exceeded the Swedish occupational exposure limit of 150 mg/m^3 on occasion, usually in the winter (Eriksson et al., 1997). Exposures in the saw house typically were less than one quarter of the Finnish occupational exposure limit (570 mg/m^3) (Rosenberg et al., 2002). Lung function tests suggested chronic rather than acute reactions in airways (Eriksson et al., 1997).

Animal Toxicity: Little information was available regarding toxicity of the pinenes in animal models. The treatment of primary chick embryo liver cells with α-pinene resulted in increased production of porphyrin, predominantly the copro- and protoporphyrins (Bonkovsky et al., 1992; cited by HSDB, 2002b). In fowls, β-pinene caused leukemic changes in the plasma and deviations in avian plasma proteins, accompanied by erythroblastosis (Clayton and Clayton, 1981 – 1982; cited by HSDB, 2000c).

Chemical Disposition, Metabolism, and Toxicokinetics: Both α- and β-pinene are readily absorbed through the pulmonary system, the skin, and the intestines (Clayton and Clayton, 1981-1982; cited by HSDB, 2002c; Budavari, 1996; cited by HSDB, 2002b). Several human exposure studies were identified where males were exposed to α-pinene for two hours in an exposure chamber while performing light work. Pulmonary uptake averaged about 59% of the exposure concentration. Absorption uptake for α-pinene increased linearly with concentration (Falk et al., 1990; cited by HSDB, 2002b). Respiratory elimination represented approximately 7.6% of the total uptake. Urinary excretion of α-pinene, as measured by the metabolite verbenol, was 3.8% and 1.7% at 10 and 450 mg/m^3, respectively, at four hours (Levin et al., 1992; cited by HSDB, 2002b). The mean blood concentration was linearly related to the inhaled concentration at the end of the exposure (Falk et al., 1990; cited by HSDB, 2002b). Elimination of α-pinene from the blood was triphasic with half-lives ($t_{1/2}$) for the (+)enantiomer of 4.8, 39, and 695 minutes. Elimination of the (-)enantiomer was somewhat more rapid overall, with $t_{1/2}$ of 5.6, 40, and 555 minutes. Urinary elimination of unchanged α-pinene was less than 0.001% (Falk et al., 1990; cited by HSDB, 2002b). Percutaneous absorption was demonstrated through immersion of pigs and a single human subject in a diluted pine oil solution (150 mL of pine oil diluted to 450 mL). α- And β-pinene were detected within 20 minutes of immersion and reached maximum

levels within 50 to 75 minutes in exhaled breath. Pinenes were still detectable 24 hours after exposure (further details not provided) (Opdyke, 1979; cited by HSDB, 2002c).

Synergistic/Antagonistic Interactions: Male rats treated with β-pinene demonstrated increased heptachlor mortality and benzo[a]pyrene hydroxylation while showing no change in hexobarbital sleeping time or parathione mortality (Sperling et al., 1972; cited in HSDB, 2002c).

Carcinogenicity: In a review of chemicals used in wood and associated industries, α- and β-pinene were excluded from further evaluation based on insufficient available evidence for evaluation from previous, current, or planned tests (Sigman et al., 1984).

Anticarcinogenicity: Some studies have examined the cancer chemopreventative activities of several of the monoterpenes. In an *in vitro* assay, α-pinene inhibited the growth of viral Ha-*ras*-transformed rat liver epithelial cells (WB-*ras* cells) at concentrations ranging from 0.25 to 2.5 mM (Ruch et al., 1994). However, *in vivo*, α-pinene failed to demonstrate significant chemopreventative action in the DMBA-induced rat mammary carcinogenesis (doses not provided) (Russin et al., 1989).

Other Data: The ability of several of the monoterpenes, either alone or in combination, to improve dermal penetration of various pharmaceuticals was studied in three separate investigations. α-Pinene, alone, doubled the permeability coefficient of an aqueous solution of 5-flurouracil (Williams and Barry, 1991). In combination with β-pinene and α-terpineol, there was a synergistic effect with both 1,8-cineole and *d*-limonene to enhance the permeation of indomethacin (Huang et al., 1999). α-Pinene had little effect on the penetration of either chlorpromazine or haloperidol (Almirall et al., 1996).

10.2 3-Carene [CAS RN 74806-04-5]

Searches for 3-carene on TOXLINE and PUBMED resulted in five unique studies regarding the chemical. For the most part, the studies considered potential occupational exposures to monoterpenes (α- and β-pinene and 3-carene) found in sawmill and soldering fumes using colophony flux (Söderkvist, 1987; Sèoderkvist, 1987). Several studies investigating the chronic effects of occupational exposures to monoterpenes found in sawmill fumes were included in this set of citations. In a cross-sectional study of 38 workers in four joinery shops, data were collected through personal air sampling, biological monitoring of urinary metabolites, and standardized questionnaires and interviews. Personal exposures ranged from 10 to 214 mg/m^3, exceeding the Swedish occupational exposure limit of 150 mg/m^3 on occasion (Eriksson et al, 1997). This and other studies (Söderkvist, 1987; Sèoderkvist, 1987) concluded that exposures to sawmill fumes resulted in chronic lung function impairment. In the Eriksson et al. study (1997), a significant reduction was found in the pre-shift lung function values relative to a local reference group, even when smokers and former smokers were excluded, contributing to the conclusion that acute airway reactions were not responsible for the lung function impairment. Furthermore, spirometry testing indicated a reduction in forced expiratory volume (FEV), forced vital capacity (FVC), and maximum mid-expiratory flow (MMF). Increases in the closing volume percentage

and slope of the alveolar plateau (phase III) were observed prior to exposures on Monday morning with a single-breath nitrogen washout (Söderkvist, 1987).

A single toxicokinetic study focused on 3-carene was identified. Human volunteers were exposed to 10, 225, and 450 mg/m^3 3-carene in an exposure chamber for three (two-hour) exposures. Pulmonary uptake of 3-carene was in the 70% range for the two higher exposure levels and increased linearly with exposure levels. Approximately 3% of the total uptake was eliminated unchanged via the lungs, with less than 0.001% eliminated through the urine. A long half time in the blood was noted, suggesting a high affinity for adipose tissues (Falk et al., 1991).

10.3 Limonene [CAS RN 138-86-2 or 5989-27-5]

d-Limonene is found in turpentine in concentrations ranging from 0 to 20%. The search strategy used for the PUBMED database for limonene used the terms limonene or 138-86-3 or 5989-27-5 (the CAS RN specific for *d*-limonene) with neoplasms or chronic or long term resulted in 103 citations, 44 of which were considered further. A similar strategy was used for TOXLINE, but restricting the search to *d*-limonene.

Human Exposure: Occupational exposures (inhalation or dermal) are likely during the production, formulation, transportation, and use of limonene. Concentrations of limonene have been detected in the range of 25 to 130 μg/m^3 and 5 to 1700 μg/m^3 in the vulcanization areas of a shoe sole and tire retreading factories, respectively. One survey detected limonene in the air of an office building (43 μg/m^3) in 1987. In a separate survey (1981 to 1982), limonene was detected qualitatively in two preschools in Stockholm. The general population may potentially be exposed to limonene through releases into the atmosphere from natural sources or from common consumer products. Limonene is naturally occurring in some foods, or may be added as a flavor or fragrance ingredient (HSDB, 2002a). *d*-Limonene is considered to be generally regarded as safe (GRAS) by the FDA for use as a flavor or fragrance (Flamm and Lehman-McKeeman, 1991; cited by HSDB, 2002a).

Human Toxicity: Limonene is considered to be a mild local irritant and skin sensitizer (Gosselin et al., 1984; cited by HSDB, 2002a). The liquid may be irritating to the eyes and the gastrointestinal tract if ingested (U.S. Coast Guard, 1984-1985; cited by HSDB, 2002a). If ingested in sufficient quantities, limonene may cause albuminuria and hematuria (Gosselin et al., 1984; cited by HSDB, 2002a).

A single case-control study was identified that evaluated citrus consumption and its impact on squamous cell carcinoma (SCC) in an older population in the Southwestern United States. Weekly consumption of citrus fruit and citrus juice was reported by 64.3% and 74.5% of the respondents, respectively. Peel consumption was not uncommon, with 34.7% of all subjects reporting citrus peel use. The greatest protection against SCC was derived from the actual consumption of the citrus peels (OR 0.66; 95% CI = 0.45 to 0.95). In addition, the study demonstrated a dose-response relationship between the higher consumption rates of citrus peel

and the degree of risk reduction. Citrus peel consumption represents a major source of dietary limonene and may have a potential protective effect in relation to SCC (Hakim et al., 2000).

Limonene was tested in a Phase I clinical trial to assess the toxicity, maximum tolerated dose, and pharmacokinetics in patients with advanced cancer. Patients (32) with refractory solid tumors completed 99 courses of *d*-limonene (0.5 to 12 g/m^2/d, orally, as reported) in 21-day cycles. Ten additional breast cancer patients received 15 cycles of *d*-limonene at 8 g/m^2/d. Eight g/m^2/d was determined to be the maximal tolerated dose, causing nausea, vomiting, and diarrhea. A partial response, lasting 11 months, was observed in one breast cancer patient dosed at 8 g/m^2. Three of the colorectal carcinoma patients progressed to prolonged stable disease (Vigushin et al., 1998).

Chemical Disposition, Metabolism, and Toxicokinetics: One study suggested that the monoterpenes are poorly resorbed (as cited) from the gastrointestinal tract, with accumulation in the lipophilic body compartments. Metabolism of the monoterpenes occurs within the lipophilic body compartments. Elimination occurs primarily through the kidneys (Koppel et al., 1981; cited by HSDB, 2002a). Excretion of [^{14}C]-labeled limonene administered orally to animals (species and numbers not provided) and humans occurred predominantly through the kidneys (75 to 95%), with only 10% of the radioactivity recovered in the feces within two to three days (Kodamar et al., 1976; cited by HSDB, 2002a). The major metabolites observed in the urine of limonene-treated animals are species specific: perillic acid-8,9-diol in rats and rabbits; perillyl-*β*-d-glucopyranosiduronic acid in hamsters; *p*-menth-1-ene-8,9-diol in dogs; and 8-hydroxy-*p*-menth-1-en-9-yl-*β*-d glucopyranosiduronic acid in guinea pig and humans (Kodama et al., 1976; cited by HSDB, 2002a). Additional urinary metabolites for the dog and rat (2-hydroxy-*p*-menth-8-en-7-oic acid, perillyl glycine, perillyl-*β*-D-glucopyranosiduronic acid, *p*-mentha-1,8-diene-6-ol, and probably *p*-menth-1-ene-6,8,9-triol) were reported by NTP (1990; cited by HSDB, 2002a).

Animals (additional details not provided) were exposed to radioactive limonene in a foam bath to study percutaneous uptake. Maximum blood levels of limonene were reached after ten minutes of exposure. The concentrations achieved in the blood were proportional to the surface area of the exposed skin (Schafer et al., 1982; cited by HSDB, 2002a).

Inhalation studies of *d*-limonene (10, 25, or 450 mg/m^3, for two hours) in human volunteers demonstrated a relative pulmonary uptake in the range of 70%. Although no irritative symptoms or symptoms related to central nervous system impacts were observed, a reduction in vital capacity was noted for the high-level limonene group (Falk-Filipsson, 1993; cited by HSDB, 2002a).

Plasma and urine samples were collected from treated patients involved in clinical trials of limonene in the United Kingdom as a cancer therapeutic (0.5 to 12 g/m^2/day, 21-day cycles). Analysis of these samples indicated that limonene was rapidly converted to hydroxylated or carboxylated derivatives. Five major metabolites were detected in the plasma extracts: limonene-1,2-diol, limonene-8,9-diol, perillic acid, an isomer of perillic acid, and dihydroperillic acid. Identified urinary metabolites included: glucuronides of isomers of perillic acid, dihydroperillic

acid, limonene-8,9-diol, and monohydroxylated limonene (Poon et al. 1996). In a separate Phase I study, peak plasma concentrations (C_{max}) were measured for the predominant circulating metabolites: perillic acid (C_{max} = 20.7 ± 13.2 to 71 ± 29.3 μM), dihydroperillic acid (C_{max} = 16.6 ± 7.9 to 28.1 ± 3.1 μM), limonene-1,2-diol (C_{max} = 10.0 ± 8 to 20.7 ± 8.6 μM), and uroterpenol (C_{max} = 14.3 ± 1.5 to 45.1 ± 1.8 μM). An isomer of perillic acid was identified, but not quantified. Intratumor *d*-limonene and uroterpenol levels were higher than the corresponding plasma levels. *d*-Limonene was well tolerated in cancer patients at doses that may have clinical activity (Vigushin et al., 1998).

As with turpentine, there is anecdotal evidence that limonene may cause allergic reactions in humans. One dental surgery patient presented with intense swelling of the tongue, lips, and gingival mucosa. The diagnosis was a hypersensitivity reaction to a dental preparation that contained peppermint oil, of which limonene was a component (Dooms-Goossens et al., 1977; cited by HSDB, 2002a). Three cases of allergic contact dermatitis were traced to dipentene (limonene) used in the same brand of honing oil (Rycroft, 1980; cited by HSDB, 2002a). Limonene failed to cause irritation in a closed (as cited) patch test in 25 individuals (Opdyke, 1979; cited by HSDB, 2002a).

Acute Animal Toxicity: When adult male and female Sprague Dawley rats were given a single oral dose of limonene (0, 0.1, 0.3, 1, or 3 mmol [0, 14, 41, 136, or 409 mg/kg]) in corn oil, a dose response was observed for acute exacerbation of hyaline droplets. The droplets were graded according to their size, eosinophilic intensity, and the number of tubules with droplets. The mean score for control animals was three. No hyaline droplet accumulation was observed in the male rats dosed with 0.1 mmol; 3 mmol-dosed males were given a score of ten. At 24 hours after dosing, the renal concentration of *d*-limonene equivalents (as cited) was 2.5 fold higher in the males dosed with 3 mmol/kg limonene than in the females of the same dose group. Forty percent of the renal *d*-limonene equivalents found in the male rats were associated with proteins having a molecular weight of about 20,000. The protein was identified as α_{2u}-globulin by amino acid sequencing (Lehman-McKeeman et al., 1989; cited by HSDB, 2002a).

Short-term and Subchronic Effects: Several short-term and subchronic toxicity studies were identified. Dietrich and Swenberg (1991; cited by HSDB, 2002a) dosed male NCI Black Reiter rats with 1650 mg *d*-limonene/kg body weight by gavage for four days. Twenty-four hours after the final dose, animals were sacrificed and the kidneys removed and sectioned to assess nephrotoxicity. *d*-Limonene failed to induce hyaline droplet formation or α_{2u}-globulin formation under these experimental conditions. The authors concluded that the presence of α_{2u}-globulin was necessary for the development of renal disease in male rats exposed to *d*-limonene. Webb et al. (1990) gavaged dogs twice a day with doses approaching the ED_{50} for emesis (0.12 or 1.2 mL/kg body weight [100 or 1000 mg/kg] per day) for six months. Although linear regression analysis revealed a positive dose-related trend in both the absolute (male and female) and relative kidney weights (female only), no histopathological changes associated with the change in kidney weights could be found, nor was there any evidence of hyaline droplet accumulation or other signs of hydrocarbon-induced nephropathy.

In rats dosed with *d*-limonene for seven days, an inhibition of cholesterol biosynthesis in the small intestines was observed without any significant effect on the secretion of radiolabeled cholesterol into the bile or feces (NTP, 1990; cited by HSDB, 2002a). Ariyoshi et al. (1975; cited by HSDB, 2002a) found that repeated oral dosing (400 mg *d*-limonene/kg body weight for 30 days) resulted in a decrease in both plasma and liver cholesterol, along with alterations in the fatty acids of liver phospholipids in rats. There was a concomitant increase in aminopyrine demethylase and aniline hydroxylase (26 and 22% respectively). A single oral dose of *d*-limonene (ranging from 200 to 1200 mg/kg body weight) failed to affect enzyme levels. A six-month oral dosing study (1.2 to 3.6 mL *d*-limonene/kg per day), causing frequent vomiting and nausea in dogs, also resulted in an overall decrease in body weight along with decreased blood sugar and cholesterol levels (Tsuji et al., 1975; cited by HSDB, 2002a). Intravenous injections of *d*-limonene directly into the common bile duct of dogs increased the perfusion pressure of the sphincter of Oddi (NTP, 1990; cited by HSDB, 2002a).

Renal effects in a previously conducted 91-day oral dosing study of *d*-limonene in mice and rats were evaluated by Kanerva and Alden (1987). Dose-related decreases were observed in both the absolute and relative weight gains of the experimental animals. The highest dose (2400 mg/kg) was lethal to nine out of ten female rats. Renal alterations were observed only in the male rats. Histopathological examination of the kidneys revealed cytoplasmic basophilia of the proximal convoluted tubule cells, tubular hyperplasia or atrophy, fibrosis of Bowman's capsule, and an interstitial fibrolymphocytic response. The severity of effects was dose-related, with the exception of the highest dose (2400 mg/kg), which was more similar to the effects found in the 150 mg/kg dose group. Occasional foci of proximal convoluted tubule epithelial cell necrosis were seen in all treated rats. With a single exception, granular casts in the outer medulla were observed in all animals surviving to the end of the study. The authors failed to observe hyaline droplet accumulation within the cytoplasm of the proximal convoluted tubule epithelial cells.

Reproductive and Teratologic Effects: An increase in abnormal bone formation (fetuses) and a decrease in body weight gain (male offspring) was noted for mice dosed orally with 2363 mg/kg *d*-limonene on gestation days seven through twelve; maternal body weight gain was also depressed (Kodame et al., 1977; cited by HSDB, 2002a).

Carcinogenicity: The International Agency for Research on Cancer (IARC) has indicated that there is inadequate evidence for carcinogenicity of *d*-limonene in humans. There is, however, sufficient evidence for carcinogenicity in experimental animals. In their overall evaluation, the working group concluded that *d*-limonene produces renal tubular tumors in male rats by the non-DNA reactive α_{2u}-globulin-associated responses that are not relevant to humans. Therefore, IARC classified *d*-limonene as a Group 3 chemical: not classifiable as to its carcinogenicity to humans (IARC, 1999; cited by HSDB, 2002a).

d-Limonene was tested by the NTP for general and genetic toxicity and carcinogenicity in male and female rats (F344/N) and mice ($B6C3F_1$) (NTP TR-347, 1990). Rats received 150 to 2,400

mg *d*-limonene/kg (1.10 to 17.62 mmol/kg) and mice received 125 to 2,000 mg *d*-limonene (99% pure)/kg (0.917 to 14.68 mmol/kg) by oral gavage for 13 weeks. No gross histopathological observations were seen in female rats or mice of either sex. However, male rats showed a compound-related increase in nephropathy. Degeneration of epithelial cells in the convoluted tubules, granular casts (no additional information provided by NTP to characterize the casts) in the outer stripe of the medulla, and epithelial regeneration was noted. These lesions were characteristic of the hyaline droplet nephropathy associated with an accumulation of liver-generated α_{2u}-globulin in the cytoplasm of tubular epithelial cells. No evidence of carcinogenic activity was found in either male or female B6C3F1 mice dosed with 250 or 500 mg/kg or 500 or 1000 mg/kg, respectively, by oral gavage five days a week for 103 weeks. These studies also failed to demonstrate carcinogenic activity in female F344/N rats at 300 or 600 mg/kg, for the same period of time. However, clear evidence for carcinogenic activity was shown in male F344/N rats (75 or 150 mg/kg by oral gavage five days a week for 103 weeks), as demonstrated by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas in both groups of exposed animals.

Initiation/Promotion Studies: Elegbede et al. (1986b) investigated the promotional capability of both orange peel oil and *d*-limonene, a major component of orange peel oil, in a two-stage carcinogenesis model. Orange peel oil and *d*-limonene (doses not provided) were applied topically to DMBA-initiated mouse skin. While orange peel oil was found to be a very weak promoter of both skin papillomas and carcinomas, the effects were thought to be due to one or more of the minor components of the oil, as *d*-limonene alone had no promotional effects.

Anticarcinogenicity: The anticarcinogenic properties of limonene have been tested in a wide variety of chemically induced animal model systems. Limonene was frequently added to the diets of the animals for these studies. Elson et al. (1988) found that *d*-limonene reduced the average number of rat mammary carcinomas when fed either prior to initiation or during the promotion/progression stages of carcinogenesis. When *d*-limonene (1000 or 10,000 ppm) was added to the diets one week prior to initiation with DMBA, there was a significant reduction in mammary carcinogenesis at both levels at week 27, primarily due to an increase in latency. At 18 weeks, there was a 72% reduction in mammary tumors in the limonene treated animals relative to the positive control animals (Elegbede et al., 1984). Time to first tumor was only affected if limonene was fed prior to initiation (Elson et al., 1988). In (W/FuXF344)F2 rats induced with DMBA and then assigned to diets with or without limonene at the time of first observable tumor, there was a highly significant regression in the first tumors and an inhibition of the formation of subsequent tumors in the group receiving 10% *d*-limonene in their diets (Elegbede et al., 1986a). In a similar fashion, Chander et al. (1994) found that the addition of limonene (10% or 5%) to the diet of animals induced with *N*-nitroso-*N*-methylurea (NMU) resulted in tumor regression in 100% or 50% of the animals, respectively. When combined with 4-hydroxyandrostrenedione (12.5 mg/kg, producing regression of tumors in 75% of initiated animals) 5% limonene increased tumor regression to 83.3% of treated animals.

d-Limonene was also effective as an anticarcinogen in the 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)- and *N*-nitrosodiethylamine (NDEA)-induced forestomach and lung neoplasia models (Wattenberg and Coccia, 1991; el-Bayoumy et al, 1996; Giri et al., 1999). *d*-Limonene inhibited pulmonary adenoma formation (about 35% reduction) and occurrence of forestomach tumors (about 60% reduction) when given orally either 15 minutes or one hour before NDEA or NNK, respectively (Wattenberg et al., 1989; Wattenberg and Coccia, 1991). When added to the diet one week prior to initiation with NNK, *d*-limonene significantly reduced the number of lung tumors per mouse. However, there was no measurable effect on lung tumor incidence (el-Bayoumy et al., 1996). *d*-Limonene completely inhibited NDEA- and NDEA plus phenobarbital-induced overexpression of both c-*jun* and c-*myc* mRNA and protein in AKR mice (Giri et al., 1999).

In *N*-methyl-*N′*-nitrosoguanidine (MNNG)-induced gastric cancer in Wistar rats, animals consuming diets containing 2%, but not 1%, limonene had a significant decrease in the incidence of gastric cancer. A significant reduction in labeling index was accompanied by a significant increase in apoptotic index. The results suggested that limonene inhibited the development of gastric cancer through an increase in apoptosis and reduction in cell proliferation (Uedo et al., 1999). Similar results were obtained in the sodium chloride-enhanced MNNG gastric cancer model. Wistar rats were exposed to MNNG for 25 weeks and then placed on a 10% sodium chloride or 10% sodium chloride plus 1% limonene diet until week 52. At the end of 52 weeks, animals exposed to MNNG and sodium chloride had significantly increased incidences of gastric cancer, labeling index, and ornithine decarboxylase (ODC) activity relative to control animals (untreated). The apoptotic index was significantly decreased in this treatment group. The animals placed on the sodium chloride plus limonene diet had a significantly reduced incidence of gastric cancer. The labeling index and ODC activity were both significantly reduced relative to the sodium chloride diet animals. There was a significant increase in the apoptotic index in animals treated with sodium chloride and limonene relative to the group on sodium chloride only. Limonene appeared to have attenuated gastric carcinogenesis enhanced by sodium chloride by increasing apoptosis and decreasing ODC activity (Yano et al., 1999).

Unlike the gastric-induced models of carcinogenesis, when pancreatic cancer was induced in Syrian golden hamsters by five weekly injections of *N*-nitrosobis (2-oxopropyl)amine (BOP), followed by 26 weeks on a *d*-limonene-containing diet, there was a significant reduction in the number of pancreatic carcinomas, but this occurred without a significant increase in apoptotic index. There was also a significant decrease in bromodeoxyuridine (BrdU) labeling. The reduction in carcinomas in this instance occurred not through enhanced apoptosis, but rather, through an inhibition of cellular proliferation (Nakaizumi et al., 1997).

Other miscellaneous cancer models associated with *d*-limonene chemoprevention or chemoprotection include benzo[a]pyrene-induced skin carcinogenesis and azoxymethane (AOM)-induced aberrant crypt foci in male F344 rats. In the benzo[a]pyrene model, limonene, applied to ICR/Ha Swiss mouse skin, only partially inhibited the development of skin tumors (van Duuren and Goldschmidt, 1976). Dietary *d*-limonene combined with the AOM treatment of

rats significantly reduced aberrant crypt foci ($p<0.01$), number of aberrant crypts/colon ($p<0.001$), and the number of aberrant crypts per foci ($p<0.001$). In addition, there was a smaller number ($p<0.001$) of silver stained nucleolar organizer region proteins in the limonene treated animals (Kawamori et al., 1996).

Modulation of several cellular, metabolic, and molecular activities have been associated with terpene exposures, such as the inhibition of coenzyme Q synthesis, the induction of mannose-6-phosphate/insulin-like growth factor II (IGF II) receptor, and the induction transforming growth factor-β (TGF-β) (Gould, 1995). *d*-Limonene appears to be active in both the initiation phase and the promotion/progression phase of carcinogenesis (Elson et al., 1988). Elegbede et al. (1993) reported that limonene (5% in the diet) resulted in a doubling in hepatic glutathione-S-transferase activity based on the substrates 1-chloro-2,4-dinitrobenzene and 3,4-dichloronitrobenzene; no significant changes were found for the substrate 1,2-epoxy-3-(*p*-nitrophenoxy) propane. Limonene also increased the activity of 3-MC- and phenobarbital-inducible uridine diphosphoglucuronosyl transferase isozymes. Maltzman et al. (1991; cited by HSDB, 2002a) indicated that *d*-limonene modulated cytochrome P450 (CYP450), specifically the CYP2B and 2C families, and epoxide hydrolase activities. Wistar-Furth rats maintained on limonene-containing diets formed 50% less adducts in livers, spleens, kidneys and lungs, relative to control animals. However, there was no difference in the circulating levels of DMBA and/or its metabolites between the DMBA-treated animals and DMBA plus limonene treated animals. A 2.3 fold increase in DMBA and/or its metabolites was found in the urine of animals on the limonene-containing diet. These results were supported by studies by Crowell et al. (1992; cited by HSDB, 2002a), who showed that animals fed a 5% limonene diet had a two-fold increase in the excretion of radioactive [^{3}H]-DMBA.

Elson and Yu (1994) suggest that *d*-limonene suppressed hepatic 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase activity. HMG-CoA is involved in cholesterol synthesis, supporting studies reporting a decrease in serum cholesterol levels. In mevinolin inhibition of HMG-CoA reductase activity, there is a depletion of intermediate products required for post-translational modification of proteins resulting in a reduction of cell proliferation, another feature reported in anticarcinogenicity studies with *d*-limonene.

Crowell and Gould (1994) proposed that the effectiveness of *d*-limonene during the promotion/progression phase was probably due to inhibition of post-translational isoprenylation of growth-controlling small G proteins, such as p21*ras*. Gould et al. (1994) tested this hypothesis by examining two types of mammary tumors: direct retroviral gene transfer of v-Ha-*ras* into mammary parenchyma cells and NMU-induced mammary carcinomas. While *d*-limonene was effective in inhibiting formation of mammary carcinomas (retrovirally induced), increasing the latency, and reducing the frequency of mammary carcinomas relative to control treatment, it did not alter the percentage of carcinomas with active *ras* in the NMU-treated carcinomas. Carcinomas without the activated *ras* were prevented to the same extent as those with an activated oncogene.

Genotoxicity: *d*-Limonene (doses not provided) was not mutagenic in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537), or in mouse lymphoma L5178Y/TK$^{+/-}$ assay, and did not induce chromosomal aberrations or sister chromatid exchanges (SCE) in cultured Chinese hamster ovary (CHO) cells. All assays were conducted in the presence and absence of metabolic activation (NTP TR-347, 1990).

Turner et al. (2001) tested the mutagenic potential of *d*-limonene in male lacI transgenic (Big Blue) rats. In studies where male Big Blue rats were exposed for ten consecutive days to doses of limonene that exceeded those used in the NTP gavage carcinogenicity bioassays, limonene failed to increase the mutation frequency in the liver or kidney, supporting a non-genotoxic mechanism of carcinogenic action.

Immunotoxicity: The allergic potential of oxidation products of *d*-limonene, (R)-(-)-carvone, *cis*- and *trans*-limonene oxide, and *cis*- and *trans*-carveal, along with air oxidized *d*-limonene, was investigated in Dunkin–Hartley guinea pigs. Although air exposed *d*-limonene was the strongest sensitizer in both the Freund complete adjuvant test and the guinea pig maximization test, (+) limonene oxide and (R)-(-)-carvone were also found to be potent sensitizers (Karlberg et al., 1992; cited by HSDB, 2002a).

The effects on T- and B-cell responses in BALB/c mice treated with *d*-limonene for 9 weeks were evaluated at week four and eight by Evans et al. (1987). Concanavalin-A, phytohemagglutinin, and lipopolysaccharide responses at week eight, but not four, were suppressed. When the mice were primed with keyhole limpet hemocyanin (KLH) prior to *d*-limonene exposure, there was a suppression of primary and secondary anti-KLH responses; when the mice were exposed to *d*-limonene prior to the KLH, there was a significant increase in the antibody response. These results were supported by histopathological examination of secondary lymphoreticular tissues. The results suggested that *d*-limonene has polyclonal activator action.

11.0 Online Databases and Secondary References

11.1 Online Databases

STN International Files

AGRICOLA	EMBASE	NTIS
BIOSIS	HSDB	RTECS
CA	LIFESCI	TOXLINE
CABA	MEDLINE	
CANCERLIT	NIOSHTIC	

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicological Research Projects	CRISP
NIOSHTIC®	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

In-House Databases
CPI Electronic Publishing Federal Databases on CD
Current Contents on Diskette®
The Merck Index, 1996, on CD-ROM

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Units And Abbreviations

°C = degrees Celsius

μg/L = microgram(s) per liter

μg/m^3 = microgram(s) per cubic meter

μg/mL = microgram(s) per milliliter

μM = micromolar

ACGIH = American Conference of Governmental Industrial Hygienists

bw = body weight

CNS = central nervous system

EPA = Environmental Protection Agency

F = female(s)

g = gram(s)

g/mL = gram(s) per milliliter

h = hour(s)

HD = high dose

HSDB = Hazardous Substances Data Bank

i.p. = intraperitoneal(ly)

kg = kilogram(s)

L = liter(s)

lb = pound(s)

LC = liquid chromatography

LC_{50} = lethal concentration for 50% of test animals

LD_{50} = lethal dose for 50% of test animals

LOD = limit of detection

M = male(s)

MD = mid dose

mg/kg = milligram(s) per kilogram

mg/m^3 = milligram(s) per cubic meter

mg/mL = milligram(s) per milliliter

min = minute(s)

mL/kg = milliliter(s) per kilogram

mm = millimeter(s)

mM = millimolar

mmol = millimole(s)

mmol/kg = millimoles per kilogram

mo = month(s)

mol = mole(s)

mol. wt. = molecular weight

NIEHS = National Institute of Environmental Health Sciences

NIOSH = National Institute for Occupational Safety and Health

NOEL = no observable effect level

nm = nanometer(s)

n.p. = not provided

NTP = National Toxicology Program

OSHA = Occupational Safety and Health Administration

PEL = permissible exposure limit

ppb = parts per billion

ppm = parts per million

p.o. = peroral(ly), *per os*

REL = relative exposure limit

s = second(s)

s.c. = subcutaneous(ly)

STEL = short-term exposure limit

TSCA = Toxic Substances Control Act

TWA = time-weighted average

wk = week(s)

yr = year(s)

Appendix: Literature Search and Identification Strategy

Database searches for turpentine were done in late February and early March 2001 in more than one session due to some early confusion of CASRNs and nomenclature (gum vs. oil). A more straightforward approach was used on March 12, 2001, to recapitulate the strategy (after results had already been examined). A search of the assigned compound was conducted on the STN biomedical (AGRICOLA, BIOSIS, BIOTECHNO, CABA, CANCERLIT, EMBASE, LIFESCI, MEDLINE, NIOSHTIC, and TOXLINE) and NTIS databases. Use of the keyword turpentine and the CASRNs for oil of turpentine (8006-64-2) and gum turpentine (9005-90-7), which is also called pine resin or pine gum, produced a set of 3211 records after duplicate removal. Before duplicate removal, I noted that only 40% of the records contained CASRNs and that only 15% of the turpentine records contained the terms "turpentine oil" or "oil of turpentine." Had the search been limited to turpentine oil and 8006-64-2, only 31% of the available literature would have been retrieved. It became apparent that turpentine was generally used for oil of turpentine and that the CASRN for gum turpentine was frequently used in indexing turpentine. For example, 44% of the "oil of turpentine/turpentine oil" records were associated with CASRNs, roughly one-sixth of which were the wrong CASRN. The 33 records for 9005-90-7 that did not include the keyword turpentine were on pine resin or pine gum.

To reduce the answer set of 3211 records, it was combined with the following keywords ("?" is a truncation symbol for an indefinite number of characters; "W" means direct proximity in the order written):

- TOXIC? OR ADVERSE?
- WEEK? OR MONTH? OR YEAR? OR SUBCHRONIC? OR CHRONIC? OR REPEATED(W)DOS?
- CANCER? OR CARCINO? OR TUMOR? OR TUMOUR? OR LEUKEM? (sometimes also leukaem?) OR LYMPHOMA? OR NEOPLAS?
- sometimes also used HYPERPLAS? OR EPIDEMIOL?

Duplicate removal gave about 800 titles, which were examined for potential repeated-dose studies. Ultimately, to confirm that no unforeseen relevant terminology had been used, the titles of all the MEDLINE and TOXLINE records downloaded from the TOXNET site into Reference Manager and examined. (MEDLINE plus TOXLINE records represented almost half of the set of 3211 records.)