Final Report on the Safety Assessment of Juniperus Communis Extract, Juniperus Oxycedrus Extract, Juniperus Oxycedrus Tar, Juniperus Phoenicea Extract, and Juniperus Virginiana Extract[1]

The common juniper is a tree that grows in Europe, Asia, and North America. The ripe fruit of *Juniperus communis* and *Juniperus oxycedrus* is alcohol extracted to produce Juniperus Communis Extract and Juniperus Oxycedrus Extract, respectively. Juniperus Oxycedrus Tar is the volatile oil from the wood of *J. oxycedrus*. Juniperus Phoenicea Extract comes from the gum of *Juniperus phoenicea*, and Juniperus Virginiana Extract is extracted from the wood of *Juniperus virginiana*. Although Juniperus Oxycedrus Tar is produced as a by-product of distillation, no information was available on the manufacturing process for any of the Extracts. Oils derived from these varieties of juniper are used solely as fragrance ingredients; they are commonly produced using steam distillation of the source material, but it is not known if that procedure is used to produce extracts. One report does state that the chemical composition of Juniper Communis Oil and Juniperus Communis Extract is similar, each containing a wide variety of terpenoids and aromatic compounds, with the occasional aliphatic alcohols and aldehydes, and, more rarely, alkanes. The principle component of Juniperus Oxycedrus Tar is cadinene, a sesquiterpene, but cresol and guaiacol are also found. No data were available, however, indicating the extent to which there would be variations in composition that may occur as a result of extraction differences or any other factor such as plant growth conditions. Information on the composition of the other ingredients was not available. All of the Extracts function as biological additives in cosmetic formulations, and Juniperus Oxycedrus Tar is used as a hair-conditioning agent and a fragrance component. Most of the available safety test data are from studies using oils derived from the various varieties of juniper. Because of the expected similarity in composition to the extract, these data were considered. Acute studies using animals show little toxicity of the oil or tar. The oils derived from *J. communis* and *J. virginiana* and Juniperus Oxycedrus Tar were not skin irritants in animals. The oil from *J. virginiana* was not a sensitizer, and the oil from *J. communis* was not phototoxic in animal tests. Juniperus Oxycedrus Tar was genotoxic in several assays. No genotoxicity data were available for any of the extracts. Juniperus Communis Extract did affect fertility and was abortifacient in studies using albino rats. Clinical tests showed no evidence of irritation or sensitization with any of the tested oils, but some evidence of sensitization to the tar. These data were not considered sufficient to assess the safety of these ingredients. Additional data needs include current concentration of use data; function in cosmetics; methods of manufacturing and impurities data, especially pesticides; ultraviolet (UV) absorption data; if absorption occurs in the UVA or UVB range, photosensitization data are needed; dermal reproductive/developmental toxicity data (to include determination of a no-effect level); two genotoxicity assays (one in a mammalian system) for each extract; if positive, a 2-year dermal carcinogenicity assay performed using National Toxicology Program (NTP) methods is needed; a 2-year dermal carcinogenicity assay performed using NTP methods on Juniperus Oxycedrus Tar; and irritation and sensitization data on each extract and the tar (these data are needed because the available data on the oils cannot be extrapolated). Until these data are available, it is concluded that the available data are insufficient to support the safety of these ingredients in cosmetic formulations.

INTRODUCTION

Common juniper is a short to medium height tree that grows wild in many parts of Europe, Asia, and North America. The safety of the following cosmetic ingredients (from various species of juniper) is reviewed in this report: Juniperus Communis Extract (fruit extract), Juniperus Oxycedrus Extract (fruit extract), Juniperus Oxycedrus Tar (from wood), Juniperus Phoenicea Extract (gum extract), and Juniperus Virginiana Extract (wood extract). Because similarities regarding the composition of Juniper Berry Oil and Juniper Extract (fruit extract) have been identified in the published literature (e.g., Juniper Berry Oil and Juniper Extract, from *Juniperus communis* L. ssp. *nana Syme*, have the same qualitative composition), data on Juniperus Communis Oil are included in this report for use in the safety assessment of Juniperus Communis Extract. Similarly, data on Juniperus Virginiana Oil will be included for use in the safety assessment of Juniperus Virginiana Extract. The safety of Juniperus Communis Oil and Juniperus Virginiana Oil is not being evaluated in this report because both are used only as fragrance ingredients in cosmetics. According to the Cosmetic Ingredient Review (CIR) Procedures, all fragrance ingredients shall be excluded from the CIR because their safety is being determined by the Research Institute for Fragrance Materials (RIFM).

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CHEMISTRY

Chemical and Physical Properties

As noted above, common juniper is a short to medium height tree that grows wild in many parts of Europe, Asia, and North America. *J. communis* is the most common North American variety. Berries from the juniper tree contain 0.5% to 1.55% of an essential oil (Rafique, Hanif, and Chaudhary 1993). Definitions of the Juniper Extracts and Tars reviewed in this report, as well as Juniper Oils (data to be used in safety assessment of Juniper Extracts), are listed below.

Juniperus Communis Extract

Juniperus Communis Extract (CAS No. 84603-69-0) is an extract of the ripe fruit of the juniper, *J. communis*. Other names for this extract are Extract of Juniper, Extract of Juniperus Communis, and Juniper Extract (Wenninger and McEwen 1997).

Juniper Oils (from berries)

Juniperus Communis Oil (CAS No. 73049-62-4) is the volatile oil obtained from the berries of *J. communis*. It is also known as Oil, Essential, Juniper; Oil of Juniper (Wenninger and McEwen 1997); and Juniper Berry Oil (Research Institute for Fragrance Materials 1976; Lewis 1993a). Juniperus Communis Oil is a yellow liquid with a density of 0.865 and a boiling point of 120°C. It is soluble in alcohol (Grant 1972). Information from other sources indicates that the oil of *J. Communis* L. and *J. communis* L. var. *erecta* is a colorless to faintly greenish (or yellowish) liquid with a characteristic odor and aromatic bitter taste. Each oil is soluble in mineral oil and most fixed oils, but is insoluble in glycerin and in propylene glycol (Lewis 1993a; Committee on Food Chemicals Codex 1996). The oil of *J. Communis* L. var. *erecta* tends to polymerize on long storage (Committee on Food Chemicals Codex 1996).

The following physicochemical properties have been determined for another species of Juniper Oil (*Juniperus excelsa* M.B.): color (greenish yellow to yellow), specific gravity (0.8349 at 20°C), and refractive index (1.476 at 20°C) (Rafique, Hanif, and Chaudhary 1993). The chemical composition of this species of Juniper Berry Oil is discussed in Analytical Methods/Composition/Impurities. The requirements for food grade Juniper Berry Oil are also included in this section.

Juniperus Oxycedrus Extract

Juniperus Oxycedrus Extract is an extract of the ripe fruit of *Juniperus oxycedrus*. Other names for this extract include Extract of Juniper, Extract of Juniperus Oxycedrus, and Juniper Extract (Wenninger and McEwen 1997).

Juniperus Oxycedrus Tar

Juniperus Oxycedrus Tar (CAS No. 8013-10-3) is the volatile oil obtained from the wood of *J. Oxycedrus* (Wenninger and McEwen 1997). Other names for Juniperus Oxycedrus Tar include Cade Oil; Juniper Tar; Oil of Cade; Tar, Juniper; and Tar, Juniperus Oxycedrus (Wenninger and McEwen 1997); Juniper Tar Oil and Oleum Cadium (Lewis 1993b); Empyreumatic Oil of Juniper; Oil of Juniper Tar; Haarlem Oil; Harlem Oil; Tilly Drops; Holland Balsam; Silver Drops; Silver Balsam; Kaparlem; and Caparlem (Budavari 1989). It is described as an alcohol-soluble, yellow oil with a density of 0.980 to 1.055 (Lewis 1993b). According to another source, Juniper Tar is a dark brown, viscous liquid with a smoky odor and acrid, slightly aromatic taste. It has a refractive index of 1.510 to 1.530 and is soluble in the following solvents: very slightly soluble in water; soluble in ether, chloroform, amyl alcohol, glacial acetic acid, and oil turpentine; and partly soluble in alcohol or petroleum ether (Budavari 1989).

The filtrate of a mixture of 1 volume of Juniper Oxycedrus Tar with 20 volumes of warm water is acid to litmus (United States Pharmacopeial Convention, Inc. 1995).

Juniperus Phoenicea Extract

Juniperus Phoenicea Extract is an extract of the gum of *Juniperus phoenicea*. This extract is also known as Extract of Juniperus Phoenicea (Wenninger and McEwen 1997).

Juniperus Virginiana Extract

Juniperus Virginiana Extract is an extract of the wood of *Juniperus virginiana*. Extract of Juniperus Virginiana is another name for this extract (Wenninger and McEwen 1997).

Juniperus Virginiana Oil (from wood)

Juniperus Virginiana Oil (CAS No. 8000-27-9) is the volatile oil obtained from *J. virginiana*. Other names for Juniperus Virginiana Oil are Oils, Juniperus Virginiana (Wenninger and McEwen 1997); Cedarwood Oil, Virginia; Cedarwood Oil, American; Oils, Cedarwood; and Red Cedarwood Oil (Research Institute for Fragrance Materials, Inc. 1992).

Some of the physical properties of Juniperus Virginiana Oil are as follows: flash point (>200°F, closed cup); optical rotation (−36 to −16); refractive index at 20°C (1.502–1.510); solubility in alcohol (0.5–5 volumes); specific gravity (0.941–0.965 at 20°C; 0.939–0.963 at 25°C); and vapor pressure (~0.007 mm Hg at 20°C) (Research Institute For Fragrance Materials 1996).

Methods of Production

Juniperus Communis Oil and Extract

Juniper Berry Oil (*J. communis* L.) is obtained by steam distillation of the fruit of *J. communis* L. (Lewis 1993a).

Similarly, the essential oil of Portuguese juniper berries (*J. communis* L. ssp. *nana Syme*) is obtained by steam distillation of crushed berries. The yield of essential oil resulting from this process is 0.85% (Da Cunha and Roque 1989). According to another source, steam distillation for 2 hours removes approximately 35% of the volatile oil in junipers, and 95% of the oil is removed after 24 hours of steam distillation (Adams 1991). The alcoholic extract of *J. communis* L. ssp. *nana Syme* is prepared by percolation of crushed berries with an alcoholic-water mixture (50/50). The percolate is concentrated

under reduced pressure (Da Cunha and Roque 1989). The chemical composition of Juniper Berry Oil (*J. communis* L. ssp. *nana Syme*) and its alcoholic extract is included in Analytical Methods/Composition/Impurities.

Juniperus Oxycedrus Tar

Juniperus Oxycedrus Tar is a by-product of the distillation of *J. oxycedrus* (Lewis 1993b).

Juniperus Virginiana Oil

Juniperus Virginiana Oil can be obtained by steam distillation of the heartwood (Adams 1985).

Information on the methods of production of Juniperus Oxycedrus Extract, Juniperus Virginiana Extract, or Juniperus Phoenicea Extract was not found.

Analytical Methods/Composition/Impurities

The representative structures of some of the components/parent compounds of components of Juniper Oils mentioned in this section are included in Figure 1 (Lehninger 1975; Budavari 1989). Farnesol is not listed as a component of the Juniper Oils reviewed in this report. Its structure is included in Figure 1 in order to compare the structure of a monoterpene with that of a sesquiterpene, both of which are found in Juniper Oils.

Juniper Berry Oil and Extract

As determined by gas chromatography or gas-liquid chromatography, the chemical composition of Juniper Oil and Juniper Extract is included in Table 1. Here, Juniper Oil is defined as the essential oil of juniper berries from Portugal (*J. communis* L. ssp. *nana Syme*) (Da Cunha and Roque 1989) and the essential oil of a variety of juniper berries from Pakistan (*J. excelsa* M.B.) (Rafique, Hanif, and Chaudhary 1993). Juniper Extract is defined as the alcoholic extract of juniper berries from Portugal (*J. communis* L. ssp. *nana Syme*) (Da Cunha and Roque 1989). The oils of juniper and other forest trees can be very complex, containing hundreds of terpenoids and aromatic compounds. Occasionally, important amounts of aliphatic alcohols and aldehydes, and, more rarely, alkanes are also present (Adams 1991).

Hydrocarbons are the major components of Juniper Berry Oil (*J. communis* L. ssp. *nana Syme*). Monoterpene (mainly α-pinene and myrcene) and sesquiterpene (mainly β-caryophyllene, and δ-cadinene) hydrocarbons make up more than 30% of the oil. Though Juniper Oil and Juniper Extract (*J. communis* L. ssp. *nana Syme*) have the same qualitative composition, Juniper Extract is richer in sesquiterpene hydrocarbons. Borneol is the major oxygenated compound in Juniper Oil and Juniper Extract (Da Cunha and Roque 1989).

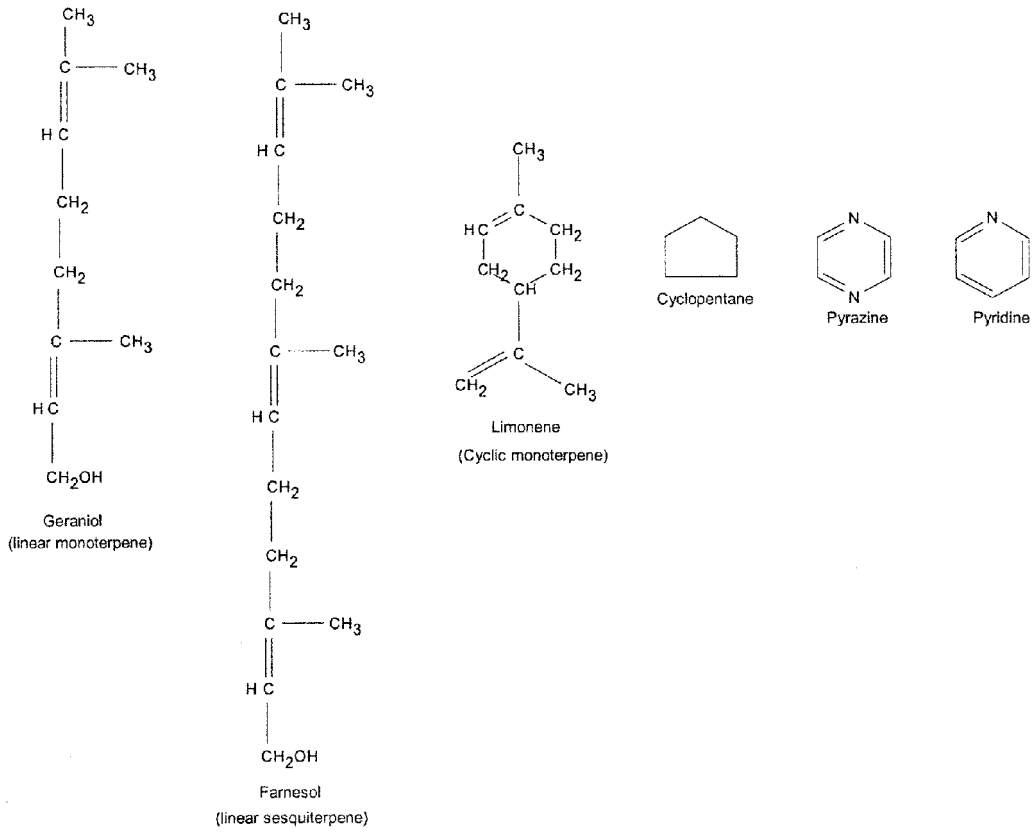

FIGURE 1
Structures of compounds and parent compounds of compounds found in Juniper Oil.

TABLE 1
Chemical composition of Juniper Extract and two species of Juniper Oil (Da Cunha and Roque 1989; Rafique, Hanif, and Chaudhary 1993)

Chemical component	Juniper Oil (*Juniperus communis* L. ssp. *nana Syme*)	Juniper Oil (*Juniperus excelsa* M.B.)	Juniper Extract (*Juniperus communis* L. ssp. *nana Syme*)
α-Pinene	20.0%	64.4%	11.0%
Fenchene	—	Traces	—
Camphene	0.2%	2.51%	0.1%
β-Pinene	1.1%	8.14%	0.7%
Sabinene	1.7%	—	1.1%
Myrcene	8.5%	12.4%	5.4%
α-Terpinene	0.2%	0.164%	0.1%
Limonene	8.7%	2.28%	5.5%
Phelandrene	—	0.072%	—
β-Phellandrene	0.3%	—	0.1%
γ-Terpinene	—	0.269%	—
t-Terpinene	0.2%	—	0.2%
p-Cymene	0.2%	0.089%	0.1%
Terpinolene	0.4%	0.082%	0.3%
α-Cubebene	0.5%	—	0.1%
α-Copaene	0.2%	—	0.6%
Camphor	0.2%	—	0.2%
Caryophyllene	—	0.12%	—
Caryophyllene Oxide	1.1%	—	1.4%
β-Caryophyllene	7.2%	—	9.8%
Terpineol-4	—	0.712%	—
Terpinene-4-ol	0.6%	—	0.7%
αHumulene	3.9%	0.105%	5.3%
Borneol	8.0%	—	8.6%
Bornyl Acetate	—	0.302%	—
α-Terpineol	0.8%	—	1.1%
Germacrene	—	0.5%	—
Germacrene D	7.0%	—	9.3%
δ-Cadinene	10.4%	—	12.8%
β-Cadinene	—	0.52%	—
γ-Cadinene	—	0.165%	—
α-Cadinol	1.3%	—	1.5%

The major components of Juniper Oil collected from the berries of *J. communis* L. in Italy were reported as follows: monoterpene hydrocarbons (α-pinene, β-myrcene, sabinene, limonene, and β-pinene) and sesquiterpene hydrocarbons (γ-elemene, γ-muurolene, β-caryophyllene, δ-cadinene, and humulene). Gas chromatography (GC) and combined gas chromatography/mass spectrometry (MS) were the analytical methods used (Bonaga and Galletti 1985). Camphene, *d*-pinene, and 1-terpineol-4 have also been listed as principal constituents of oil from the berries of *J. communis* L. (Lewis 1993a).

In addition to the compounds included in Table 1, nerol, geraniol, and carvacrol have been characterized in Juniper Berry Oil using retention time data and compound coinjection on a polar capillary column (Lawrence 1990).

The following cyclopentane derivatives have also been isolated from Juniper berries/Juniper Berry Oil: α-Campholenic aldehyde has been isolated from Juniper berries, and α-Campholenic acid, γ-campholenic aldehyde, and γ-campholenic acid have been isolated from Juniper Berry Oil. These derivatives were isolated by chemical group separation, and liquid and gas chromatography. The structures were determined by mass, infrared IR, and nuclear magnetic resonance (NMR) spectroscopy (De Rijke, Ter Heide, and Boelens 1982).

Using thin-layer chromatography (TLC), GC, MS, NMR, and IR spectroscopy, the following minor components of Juniper Berry Oil have been identified: 1-octen-3-yl acetate, methyl citronellate, bornyl acetate, campholenic aldehyde epoxide, campholenyl acetate, and campholenic aldehyde (Lawrence 1984).

Campholenic aldehyde is defined as a cyclopentane derivative in the preceding paragraph.

Lastly, two neutral compounds (diastereoisomers of 3-*p*-methene-1,2-diol), two pyrazine derivatives, and 11 pyridine derivatives were identified in crude Juniper Oil extracted with dilute acid. The extract was analyzed by GC/MS (Maurer 1994).

The requirements for food grade Juniper Berrry Oil are as follows: angular rotation (between −15° and 0°), refractive index (between 1.474 and 1.484 at 20°), specific gravity (between 0.854 and 0.879), and heavy metals (as Pb), passes test. Passing the test for heavy metals means that no darkening in color is produced in a mixture of the oil in question with water and hydrochloric acid, that is, after the mixture has been saturated with hydrogen sulfide (Committee on Food Chemicals Codex 1996).

Juniperus Virginiana Oil (Cedarwood Oil, Virginiana)

Cedarwood oil from *J. virginiana* consists predominantly of sesquiterpene hydrocarbons; α-cedrene and thujopsene are the major sesquiterpene hydrocarbon components. As determined by GC/MS, the percentages of α-cedrene, thujopsene, and other major components present in Juniperus Virginiana Oil are as follows: α-cedrene (35%), thujopsene (30%), cedrol (4%), cuparene (2%), and widdrol (2%) (Adams 1985). Juniperus Virginiana Oil has also been analyzed by IR spectroscopy (Research Institute for Fragrance Materials 1974).

Juniperus Oxycedrus Tar

The chief constituent of Juniperus Oxycedrus Tar is cadinene, a sesquiterpene (Lewis 1993b). Cresol and guaiacol, derivatives of phenol, have also been described as chief constituents of Juniper Tar (Gosselin, Smith, and Hodge 1984).

Information on the chemical composition of Juniperus Oxycedrus Extract, Juniperus Virginiana Extract, or Juniperus Phoenicea Extract was not found.

Reactivity

Juniperus Communis Oil

Juniperus Communis Oil (*J. communis* L.) emits acrid smoke and fumes when heated to decomposition (Lewis 1993a).

Juniperus Oxycedrus Tar

Juniperus Oxycedrus Tar is a combustible material, and can react with oxidizing materials (Sax 1979).

The addition of a few drops of alkaline cupric tartrate to the filtrate of a mixture of 1 volume of Juniperus Oxycedrus Tar with 20 volumes of warm water, followed by boiling of the mixture, resulted in the formation of a red precipitate (United States Pharmacopeial Convention, Inc. 1995).

Antioxidant Activity

Juniper Extract

The antioxidant activity of Juniper Extract (10% alcohol extract) was evaluated using oxidant free, low-erucic rapeseed oil. After the addition of Juniper Extract, oil samples were analyzed (acid and peroxide numbers using a titration method; thiobarbituric number using spectrophotometry; and GC to determine fatty acid composition) during 23 days of storage. The production of primary and secondary autooxidation products in the oil was inhibited by Juniper Extract (Takacsova, Pribela, and Faktorova 1995).

USE

Purpose in Cosmetics

The following ingredients reviewed in this report function as biological additives in cosmetics: Juniperus Communis Extract, Juniperus Oxycedrus Extract, Juniperus Phoenicea Extract, and Juniperus Virginiana Extract. Juniperus Oxycedrus Tar is used as a hair-conditioning agent and as a fragrance component in cosmetics (Wenninger and McEwen 1997).

Scope and Extent of Use in Cosmetics

The product formulation data on Juniper Extract, Juniper Berry Oil, Juniper Oil, and Juniper Tar submitted to the Food and Drug Administration (FDA) are included in Tables 2 and 3 (FDA 1998).

Concentration of use values are no longer reported to FDA by the cosmetics industry (FDA 1992). However, the 1984 product formulation data submitted to the FDA indicated that the maximum use concentration ranges for Juniper Extract and Juniper Tar in cosmetics have been reported as 0.1% to 1% and 1% to 5%, respectively (FDA 1984).

Cosmetic products containing Juniper Extract, Juniper Berry Oil, Juniper Oil, and Juniper Tar are applied to most parts of the body and can come in contact with the ocular and nasal mucosae. These products could be used on a daily basis, and have the potential for being applied frequently over a period of several years.

International Use

Juniperus Communis Extract and Juniperus Oxycedrus Tar are the only ingredients reviewed in this report that are listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category* (*CLS*) (Rempe and Santucci 1997). Both ingredients, which conform to the specifications of the *Japanese Cosmetic Ingredients Codex*, have precedent for use without restriction in most *CLS* categories. Additionally, both are not used in the following three *CLS* categories: eyeliner, lip, and oral preparations. Juniperus Oxycedrus Tar also is not used in bath preparations.

Juniperus sabina L. (leaves, essential oil, and its galenical preparations), which is not reviewed in this report, is included among the substances listed as prohibited from use in cosmetic products that are marketed in the European Union (Dupuis 1995). The ingredients reviewed in this safety assessment (Juniperus Communis Extract, Juniperus Oxycedrus Extract, Juniperus

TABLE 2
Product formulation data on Juniper Extract (FDA 1998)

Product category	Total no. of formulations in category	Total no. containing Juniper Extract
Bath oils, tablets, and salts	124	1
Bubble baths	200	2
Eyebrow pencil	91	1
Eyeliner	514	1
Eye shadow	506	2
Mascara	167	4
Hair conditioners	636	2
Hair sprays (aerosol fixatives)	261	1
Shampoos (non-coloring)	860	1
Blushers (all types)	238	3
Face powders	250	3
Foundations	287	4
Lipstick	790	10
Other makeup preparations	135	1
Cleansing skin care preparations	653	1
Face and neck skin care preparations (excluding shaving preparations)	263	1
Body and hand skin care preparations (excluding shaving preparations)	796	1
Night skin care preparations	188	2
Paste masks (mud packs)	255	1
Other skin care preparations	692	5
1998 Total uses of Juniper Extract		**47**

Oxycedrus Tar, Juniperus Phoenicea Extract, and Juniperus Virginiana Extract) are not included on this list of prohibited substances.

Noncosmetic Use

Juniper Oil and Juniper Extract

Juniper Berry Oil is listed among the essential oils that are generally recognized as safe for their intended use in food (21 CFR 182.20).

The *State Pharmacopoiea of the USSR* recommends juniper berries (source of Juniper Oil) for use as a diuretic. Reportedly, a diuretic effect is also exerted by essential oils from juniper berries (Mambetsadykov et al. 1990).

In its final ruling, the OTC (Over-The-Counter Drug) Advisory Review Panel for Miscellaneous Internal Drugs classified Juniper Oil as Category IISE relative to its use as a diuretic. Category II is defined as conditions under which OTC drug products are not generally recognized as safe and effective or are misbranded. In this case, the reason for this categorization of Juniper Oil is based on safety (S) as well as effectiveness (E). Similarly, in its final ruling, this Panel classified Juniper Extract as Category IISE relative to its anorectic use (FDA 1994).

Juniperus Oxycedrus Tar

Undiluted Juniper Tar has been used as a topical treatment for psoriasis (Phillips et al. 1990) and as a topical anti-eczematic medication (Budavari 1989). Juniper Tar has also been used as a topical antipruritic in chronic dermatologic disorders, such as atopic dermatitis, pruritus, and seborrhea (Gennaro 1990).

In its final ruling, the OTC (Over-The-Counter Drug) Advisory Review Panel for Hemorrhoidal Drugs classified Juniper Tar as Category I (conditions under which OTC drug products are generally recognized as safe and effective and are not misbranded) relative to its use as an external analgesic (FDA 1994).

In final rulings by the OTC Advisory Review Panel for Miscellaneous External Drugs, Juniper Tar was classified as Category IISE (conditions under which OTC drug products are not generally recognized as safe (S) and effective (E) or are misbranded) relative to the following uses: treatment of diaper rash; boil treatment; and in the treatment of dandruff, seborrheic dermatitis, and psoriasis (FDA 1994).

In proposed rules generated by the OTC Panel named in the preceding paragraph, Juniper Tar was classified as Category I (conditions under which OTC drug products are generally recognized as safe and effective and are not misbranded) relative to use in the treatment of fever blister and poison ivy, oak, and sumac. The OTC Advisory Review Panel for Topical Analgesics

TABLE 3
Product formulation data on Juniper Berry Oil, Juniper Oil, and Juniper Tar (FDA 1998)

Product category	Total no. of formulations in category	Total no. containing ingredient
Juniper Berry Oil		
Bath oils, tablets, and salts	124	1
Colognes and toilet waters	656	1
Shampoos (noncoloring)	860	2
Tonics, dressings, and other hair-grooming aids	549	2
Face and neck skin care preparations (excluding shaving preparations)	263	1
Other skin care preparations	692	2
1998 Totals		9
Juniper Oil		
Face and neck skin care preparations (excluding shaving preparations)	263	2
Body and hand (excluding shaving preparations)	796	1
Paste masks (mud packs)	255	1
1998 Totals		4
Juniper Tar		
Shampoos (noncoloring)	860	4
Tonics, dressings, and other hair-grooming aids	549	3
Paste masks (mud packs)	255	1
1998 Totals		8

also issued a proposed rule classifying Juniper Tar as Category I with respect to its analgesic, anesthetic, and antipruritic uses (FDA 1994).

GENERAL BIOLOGY

Enzyme Effects

Juniperus Virginiana Oil

Sleeping times were decreased (reduced hexabarbital-induced hypnotic effect) and hexabarbital metabolism increased in male Swiss-Webster ICR albino mice exposed to corncob bedding that had been sprayed with an ether solution of Juniperus Virginiana Oil (Cedarwood Oil, Virginiana). These effects resulted from the induction of microsomal enzymes responsible for hexobarbital metabolism. Relative liver weight was also increased (Wade et al. 1968).

Vascular Effects

Juniperus Virginiana Oil

Vasodilation was not noted after undiluted Juniperus Virginiana Oil (2 ml of neat material or in ethanol) was applied to the external ears of rabbits (Lacy, Kent, and Voss 1987).

Effects on Wound Healing

Juniper Oil

The reparative activity of Juniper Oil (juniper tree species not mentioned) was evaluated using skin wound and burn models. Compared to industrially produced sea buckthorn oil, healing times in the presence of Juniper Oil were approximately the same (23% shorter healing times for wounds and 20% shorter for burns). It was concluded that Juniper Oil exerted its optimum effect on reparative processes, causing intensive regeneration of skin wounds in animals and reducing the time required for the healing of burns. The investigators stated that this effect is manifested as faster maturation of the granulation tissue and intensive growth of the epidermal ring (Mambetsadykov et al. 1990).

Antimicrobial Activity

Juniper Oils

Three species of Juniper Oil in Arizona and Colorado (*Juniperus deppeana, Juniperus scopulorum*, and *Juniperus osteosperma*) were tested for antibacterial activity. Each oil was incubated with deer rumen inoculum (3 days of incubation with rumen fluid starch broth). Rumen inoculum was obtained by stomach pumping. (Reportedly, rumen microbial fermentation supplies approximately 50% to 70% of the energy requirements of ruminants.) Starch digestion was measured by determining the change in pH of the medium, anywhere from 6.8 for undigested media to 4.8 for control values. In starch digestion trials, *J. osteosperma* was the most inhibitory of the three oils, having reduced microbial activity ($p < .05$) below control levels at 3.0 μl of oil per ml of medium (Schwartz, Nagy, and Regelin 1980).

The antibacterial activity of Juniperus Virginiana Oil against *Bacillus subtilis* IAM 1069 and *Escherichia coli* IAM 1239 has been evaluated using the filter paper disk method. Paper disks were impregnated with the test substance and placed on filter paper for 10 minutes to remove excess. Impregnated paper disks were placed at the surface of seeded agar medium, and cultures were incubated for 24 hours. Each diameter of clear zone on the agar surface was determined. Results were negative at a test concentration of 20 mg/disk (Gocho 1991).

The antibacterial activity of Juniperus Virginiana Oil vapor has also been demonstrated using a variety of gram-positive and gram-negative bacteria (Maruzzella and Sicurella 1960).

Juniperus Virginiana Oil had no antimicrobial activity against bacteria, yeasts, and molds (Blakeway 1982).

Juniperus Virginiana Extracts

The antibacterial activity of Juniperus Virginiana Extracts (methanol and/or hexane heartwood extracts) has been demonstrated using the following bacterial strains and the agar-well diffusion assay of McChesney and Adams (1985): *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, B. subtilis*, and *Mycobacterium smegmatis*. Using the same assay, the antibacterial activity of Juniperus Virginiana Extracts (methanol and/or hexane bark/sapwood extracts) has been demonstrated using *S. aureus, B. subtilis*, and *M. smegmatis* (Adams 1985).

The antifungal activity of Juniperus Virginiana Extracts (methanol and/or hexane heartwood extracts) has been demonstrated using the following strains and the agar-well diffusion assay of McChesney and Adams (1985): *Cryptococcus neoformans* (yeast), *Saccharomyces cerevisiae* (yeast), *Pycnoporus sanguineus* (plant pathogen), *Aspergillus fumigatus* (filamentous fungus), and *Trichophyton mentagrophytes* (dermatophyte). Juniperus Virginiana Extracts (methanol and/or hexane bark/sapwood extracts) did not have antifungal activity for any of these strains (Adams 1985).

Juniperus Oxycedrus Tar

Juniper Tar, alone or combined with olive oil (1:1), had antibacterial activity against *Micrococcus citreus*, *Bacillus brevis*, and *Micrococcus pyogens*, but not against *Salmonella typhosa* and *Proteus morgani* (Research Institute for Fragrance Materials 1975).

The vapor of Juniper Tar (rectified, United States Pharmacopeia [USP]) had antibacterial activity against *Mycobacterium avium*. Antibacterial activity was not demonstrated against the following microorganisms: *E. coli*, *S. aureus*, *B. subtilis*, *Streptococcus fecalis*, and *S. typhosa* (Research Institute for Fragrance Materials 1975).

Juniper Tar also induced antifungal activity in 13 of 15 fungi tested (Research Institute for Fragrance Materials 1975).

TOXICOLOGY

Acute Oral Toxicity

Juniper Oils

In a study involving 10 rats, the acute oral LD_{50} for Juniper Berry Oil was >5 g/kg (Research Institute for Fragrance Materials 1976). The acute oral LD_{50} for Juniperus Virginiana Oil (Cedarwood Oil Virginia) in rats was >5 g/kg. Slight lethargy was noted (Research Institute for Fragrance Materials 1974).

Juniperus Oxycedrus Tar

The acute oral LD_{50} for Juniper Tar, determined using a group of 10 young adult Osborne-Mendel rats, was 8014 mg/kg (95% confidence limits = 6550–9770 mg/kg). The animals were fasted for approximately 18 hours prior to dosing. Toxic effects included depression and gastrointestinal irritation (Jenner et al. 1964).

Acute Dermal Toxicity

Juniperus Communis Oil

The acute dermal LD_{50} for Juniper Berry Oil in rabbits was >5 g/kg (Research Institute For Fragrance Materials 1976).

Juniperus Virginiana Oil

In an acute dermal toxicity study of Juniperus Virginiana Oil (Cedarwood Oil, Virginia) using nine rabbits, an LD_{50} of >5 g/kg was reported. The incidence of erythema/edema after dosing with 5 g/kg was as follows: slight redness (seven rabbits), moderate redness (one rabbit), slight edema (three rabbits), and moderate edema (six rabbits) (Research Institute for Fragrance Materials 1974).

Juniperus Oxycedrus Tar

The acute dermal toxicity of Juniper Tar for rabbits was also >5 g/kg (Research Institute for Fragrance Materials 1975).

Acute Parenteral Toxicity

Juniper Oil

The acute parenteral toxicity of a 10% solution of Juniper Oil (in corn oil) was evaluated using mongrel white mice (weights = 18–20 g; number not stated), guinea pigs (weights = 250–320 g; number not stated), and rabbits (weights = 2–3 kg; number not stated). The species of juniper tree from which the oil was derived was not stated. In all experiments, the results of which are summarized below, the test solution did not impair coordination of movement or cause muscle relaxation.

The following mean LD values (with range) were reported for mice dosed intraperitoneally: LD_0 = 650 (595–705) mg/kg; LD_{50} = 750 (685–815) mg/kg; and LD_{100} = 870 (838–902) mg/kg (Mambetsadykov et al. 1990). Values reported for guinea pigs dosed intraabdominally included an LD_0 of 1100 (1080–1120) mg/kg, an LD_{50} of 1200 (1170–1230) mg/kg, and an LD_{100} of 1500 (1430–1570) mg/kg (Mambetsadykov et al. 1990).

A mean LD_{50} of 700 (range = 654–746) mg/kg was reported for mice dosed intramuscularly with the test solution. For guinea pigs dosed subcutaneously, a mean LD_{50} of 1440 (1425–1455) mg/kg was reported (Mambetsadykov et al. 1990).

When the test solution was administered to rabbits (oral and intramuscular administration) in doses equivalent to 10× that considered therapeutic (therapeutic dose = 2–3 g/kg), no pronounced toxic reactions were detected (Mambetsadykov et al. 1990).

Cardiovascular and Respiratory Toxicity

The effect of Juniper Oil (juniper tree species not stated) on the cardiovascular system and respiration was evaluated using 20 rabbits that were anesthetized with urethane. Cardiovascular activity was evaluated by electrocardiogram (EKG). Arterial pressure, respiratory movements, and the EKG were recorded using a 2T-02 electrocardiograph. The EKG was recorded prior to dosing and at 15, 30, and 60 minutes and 24 hour post administration. When Juniper Oil was administered intramuscularly, as well as orally, at concentrations of 0.5%, 2.5%, and 5.0% in corn oil (dose = 1 mg/kg), hypotonia resulted. The hypotonia, described as prolonged, developed slowly (Mambetsadykov et al. 1990).

Skin Irritation

Juniperus Communis Oil

Undiluted Juniper Berry Oil did not induce skin irritation when applied to the backs of hairless mice and swine. However, after the oil (undiluted) was applied to intact or abraded skin of rabbits for 24 hours under occlusive patches, skin irritation was moderate (Research Institute For Fragrance Materials 1976).

Juniperus Virginiana Oil

Undiluted Juniperus Virginiana Oil (Cedarwood Oil, Virginia) also did not induce skin irritation when applied to the backs of hairless mice (Research Institute for Fragrance Materials 1974).

Juniperus Virginiana Oil induced neither skin irritation nor systemic toxicity when applied to the clipped, dorsal skin of 101 inbred mice (8–10 weeks old). Two applications of the test material, separated by a 7-day interval, were made; doses were not stated. Specimens of dorsal skin were obtained by biopsy three days after each application (Roe and Field 1965).

In another study, Juniperus Virginiana Oil was applied full strength, under occlusive patches, to the skin (intact or abraded) of rabbits for 24 hours. Skin irritation was moderate (Research Institute for Fragrance Materials 1974).

Juniperus Oxycedrus Tar

Skin irritation was not observed after undiluted Juniper Tar was applied to the backs of hairless mice. Undiluted Juniper Tar also was not irritating to the skin of rabbits when applied, under occlusive patches, to intact or abraded skin for 24 hours (Research Institute for Fragrance Materials 1975).

Skin Sensitization

Juniperus Virginiana Oil

The skin sensitization potential of 8% Juniperus Virginiana Oil (Cedarwood Oil, Virginia) was evaluated in an open epicutaneous test using groups of six to eight guinea pigs. The vehicle used was not indicated. Sensitization was not induced in any of the animals tested (Research Institute for Fragrance Materials 1996).

Phototoxicity

Juniperus Communis Oil

The phototoxicity of undiluted Juniper Berry Oil was evaluated using hairless mice and swine. No phototoxic effects were reported (Research Institute for Fragrance Materials 1976).

Juniperus Virginiana Oil

The phototoxicity of undiluted Juniperus Virginiana Oil (Cedarwood Oil, Virginia) was evaluated using six mice of the SKH:hairless-1 strain. The test substance was applied to the back (20 μl per 2 cm^2) of each animal, and sites were irradiated with a fluorescent blacklight (1 hour at integrated UVA of 3 W/m^2) or Xenon lamp (weighted erythema energy = 0.1667 W/m^2) 30 minutes later. Animals were examined at 4, 24, 48, 72, and 96 hours. The test substance was not phototoxic (Forbes, Urbach, and Davies 1977).

In another experiment, the phototoxicity of undiluted Juniperus Virginiana Oil was evaluated using two miniature swine, according to the procedure in the preceding paragraph. No evidence of phototoxicity was observed (Forbes, Urbach, and Davies 1977).

Juniperus Oxycedrus Tar

Juniper Tar also was not phototoxic when tested using hairless mice and swine (Research Institute for Fragrance Materials 1975).

Other Dermal Effects

Juniperus Virginiana Oil

The topical application of Cedarwood Oil can cause pigmentation (Research Institute for Fragrance Materials 1974).

GENOTOXICITY

Juniperus Oxycedrus Tar

The DNA-damaging activity of Juniper Tar (Cade Oil) was evaluated in bacterial strains using the spore rec-assay. Juniper Tar was tested with and without metabolic activation at a dose of 8 mg per disk. Test results were positive without metabolic activation, but were inconclusive with metabolic activation. For substances with positive effects in the preceding test, index numbers of DNA-damaging effectiveness (IDDs) in *B. subtilis* DNA were calculated using their dose response curves.

The IDD of 2.4 for Juniper Tar was considered low when compared to 22.0 for Mitomycin C and 148.1 for TRP-PPI (3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole). Mitomycin C and TRP-PPI were positive controls. Thus, based on the IDD calculated, Juniper Tar was classified as a weak agent (Ueno et al. 1984).

In another in vitro study, Juniper Tar was classified as mutagenic to *B. subtilis* in the rec-assay as well as in reverse mutation assays using *Salmonella typhimurium* or *E. coli* tester strain(s). Details concerning the assay procedures were not included (Takizawa et al. 1985).

Juniper Tar was applied daily for 5 days (50 mg per treatment) to mouse and human skin (in vivo). A single dose (50 mg) of Juniper Tar was also applied to human skin in an in vitro experiment. At 24 hours after the last dose, DNA was isolated from the epidermis and analyzed by ^{32}P-postlabeling. The standard ^{32}P-postlabeling procedure (Gupta, Reddy, and Randerath 1982) is a highly sensitive, nonspecific method for the detection of aromatic DNA adducts. A nuclease P_1 sensitivity-enhancement modification of this standard ^{32}P-postlabeling procedure (Reddy and Randerath 1986) was used in this study. The results of in vivo experiments indicated 12 adducts/10^8 nucleotides (0.37 fmol/μg DNA) induced by Juniper Tar in mouse skin and 8 adducts/10^8 nucleotides (0.23 fmol/μg DNA) induced in human skin. In the in vitro experiment, 18 adducts/10^8 nucleotides (0.54 fmol/μg DNA) were induced by Juniper Tar. It was concluded that Juniper Tar induced aromatic adducts when applied to human and mouse skin (Phillips et al. 1990).

The preceding results were compared with those of similar experiments using a coal tar–containing ointment used clinically in the treatment of psoriasis and crude coal tar. The procedures for in vivo and in vitro experiments on coal tar ointment were the same as those stated above; the coal tar content of the ointment was 675 μg per treatment. A single dose of crude coal tar (30 mg) was applied to mouse skin in vivo and human skin in in vitro experiments. The results of in vivo experiments on coal tar ointment indicated 17 adducts/10^8 nucleotides (0.50 fmol/μg DNA) induced in mouse skin and 7 adducts/10^8 nucleotides (0.22 fmol/μg DNA) induced in human skin. In the in vitro experiment, 6 adducts/10^8 nucleotides (0.19 fmol/μg DNA) were induced by coal tar ointment in human skin. Coal tar induced 13 adducts /10^8 nucleotides (0.38 fmol/μg DNA) in mouse skin in vivo and 12 adducts/10^8 nucleotides (0.35 fmol/μg DNA) in human skin in vitro. The investigators noted that DNA adduct formation induced by Juniper Tar in mouse skin in vivo (12 adducts/10^8 nucleotides) was similar to the number induced by coal tar (13 adducts/10^8 nucleotides) after 5 days of application. Crude coal tar typically contains 2 g/kg benzo[α]pyrene, a polycyclic aromatic hydrocarbon (PAH), and many PAHs are carcinogenic when applied to mouse skin. Furthermore, in consideration of the absence of carcinogenicity data on Juniper Tar, the investigators stated that it is expected that Juniper Tar would be active on mouse skin based on the extent of its induction of DNA adducts (Phillips et al. 1990).

The formation of DNA adducts in male Parkes mice (4–6 weeks old) treated with Juniper Tar was evaluated using the modification of the ^{32}P-postlabeling assay procedure in the preceding study. Approximately 50 mg (50 μl) of Juniper Tar were applied topically to mice (five groups of four) once daily for 5 days. Groups were killed at 1, 4, 7, 14, and 32 days after the last application, respectively, and the lungs and application sites were excised for subsequent DNA isolation. Untreated control animals were killed on days 1 and 14. As determined by autoradiography, aromatic DNA adducts formed in mouse epidermis and lungs. In the skin, the total number of DNA adducts formed decreased rapidly from 0.5 fmol/μg DNA on day 1 after the final treatment to 1/10 of this level after 7 days. Gradual removal of the remaining adducts was observed until, after 32 days, adducts were not detected. The time-course for adduct formation and removal was similar to that noted in the skin of mice treated with coal tar ointment (675 μg coal tar). Compared to the skin, a greater number of adducts was detected in lung DNA (0.64 fmol/μg DNA) from mice treated with Juniper Tar. The extent of DNA adduct formation persisted, either at or close to this number, for the duration of the experiment. Only very low numbers of adducts (<0.03 fmol/μg DNA) were detected in lung DNA from mice treated with coal tar ointment. This was in marked contrast to the DNA adduct formation detected in the lungs of mice treated with Juniper Tar (Schoket et al. 1990).

In the same study, a second experiment was conducted using human skin samples (normal skin) from four patients who had undergone mastectomy or reduction mammoplasty. The skin samples were subdivided into eight pieces (6–14 cm^2) and treated in organ culture as follows: Juniper Tar (6–7 mg/cm^2); coal tar ointment (6–7 mg/cm^2); and dithranol cream (5–8 mg/cm^2, equivalent to 50 to 80 μg dithranol/cm^2). Dithranol is a well-established tumor promoter. Skin samples were maintained in culture at 37°C for 24 hours after treatment in an atmosphere of 5% to 10% CO_2 in air, and then frozen pending DNA isolation. Duplicate samples of skin per patient received each treatment. Untreated skin control samples were maintained under identical culture conditions. Generally, background amounts of radioactivity in untreated skin samples were less than the equivalent of 0.15 fmol/μg DNA, but varied slightly between patients. Juniper Tar induced adduct numbers that were significantly greater than those noted in controls. Particularly, a mean value of 0.9 fmol/μg DNA was reported for one chromatogram autoradiograph. A slight increase in adduct numbers was noted in samples treated with coal tar ointment, and the amount of radioactivity on chromatograms of skin samples treated with dithranol was reproducibly similar to the numbers noted in untreated control samples (Schoket et al. 1990).

A third experiment involved samples of skin from 12 psoriasis patients undergoing treatment. The patients received the following treatments (total of 5 daily treatments on the arm): Juniper Tar (4 patients), coal tar ointment (2 patients), and coal tar ointment on one arm and Juniper Tar on the other (6 patients). Biopsies were taken from the arms 24 hours after the

last treatment. For each patient, one biopsy sample was taken from the treated area with psoriatic plaques, and the other was taken from an uninvolved, untreated area immediately adjacent to the treated area. Control skin samples consisted of small pieces of skin (obtained at surgery) from non-psoriasis patients. DNA was extracted from the samples and analyzed by ^{32}P-postlabeling. Each of six control samples had levels of radioactivity in the diagonal region of the chromatogram equivalent to less than 0.1 fmol/μg DNA. With the exception of one sample, all skin samples treated with coal tar ointment contained adduct numbers that were greater than 0.1 fmol/μg DNA; 0.39 fmol/μg DNA was the largest value. Nine skin samples treated with Juniper Tar had adduct numbers within the range of 0.15 to 0.36 fmol/μg DNA. The 10th sample had a total adduct number of less than 0.1 fmol/μg DNA. Regarding the patients who were treated with coal tar ointment on one arm and Juniper Tar on the other, no obvious correlation between the numbers of adducts was noted when the different skin sites were compared (Schoket et al. 1990).

The researchers concluded that the results of the above experiments (Schoket et al. 1990) provided evidence that potentially carcinogenic DNA damage was induced in human and mouse tissue by Juniper Tar and other components of the therapeutic tar preparations.

CARCINOGENICITY

The effect of cedarwood-based bedding (Juniperus Virginiana Extract is the extract of the wood of Juniperus Virginiana, a.k.a. cedarwood) on the development of tumors in mice has been evaluated using a breeding colony of C3H-A^{vy} mice (Heston 1975). Half of the new matings were maintained on three-fourths pine sawdust plus one-fourth cedar shavings, and the other half was maintained on pine sawdust only. After the resulting offspring were mated, they were maintained on the same types of bedding. Additional groups of males and females (also maintained on same types of bedding) were segregated as to sex (eight mice per cage) at the time of weaning. The segregated mice and female breeders were fed the National Institute of Health (NIH) open-formula diet and also given tap water. The actual numbers of mice involved in the study were as follows: males (56 on pine bedding, 56 on pine + cedar bedding), virgin females (54 on pine bedding, 55 on pine + cedar bedding), and female breeders (68 on pine bedding, 64 on pine + cedar bedding). Study results are summarized below.

Except for two female mice in the segregated group (one maintained on pine sawdust and one on pine sawdust + cedar shavings), all females developed mammary tumors at approximately 6 months of age. Thus, the mammary tumor incidence in females (breeding colony or virgin females) maintained on pine + cedar bedding was not significantly differerent from that in females maintained on pine bedding. Breeding males (6 months old) were necropsied along with their mates, and it was determined that these males were too young to provide data on tumor occurrence. The segregated males were necropsied at 12 months of age, and hepatomas were recorded. The hepatoma incidence in male mice maintained on pine + cedar bedding was not significantly different from that in males maintained on pine bedding. The researchers concluded that these studies did not provide evidence that the cedar shavings were carcinogenic (Heston 1975).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Juniperus Communis Extract

The antifertility and abortifacient activities of Juniperus Communis Extract (ethanolic extract) were evaluated using groups of 10 colony-bred, female Swiss albino rats (weights = 140–180 g). Two additional experiments were also conducted to confirm suspected abortifacient activity. Females were mated with mature males (three females per male) of proven fertility. After mating, two groups of female rats received oral doses of 300 and 500 mg/kg body weight, respectively, on days 1 to 7 of pregnancy. Each dose of the test substance was prepared with an equal amount of gum acacia, thoroughly mixed and suspended in distilled water. The 10 control rats were dosed with gum acacia suspension according to the same procedure. On day 10 of pregnancy, the rats were laparotomized under light ether anesthesia to determine the presence of implantation sites in both uterine horns. Wounds were sutured and, on days 14, 15, and 16, the same doses were administered only to rats with implantation sites. Rats were laparotomized on day 18 to determine abortifacient activity, and sutured rats were allowed to deliver. On day 10 of pregnancy, no implantation sites were present in 5 of 10 rats dosed with 300 mg/kg and in 8 of 10 rats dosed with 500 mg/kg. Implantation sites were observed in all control rats. Compared to controls, the average number of embryos was significantly reduced in the group dosed with 500 mg/kg. Following the administration of doses (days 14, 15, and 16) to the remaining rats with implantation sites, only three of five rats dosed with 300 mg/kg and neither of the two rats dosed with 500 mg/kg had implants on day 18. These results for rats with implantation sites were indicative of early abortions. For rats in which pregnancies continued, delivery was not possible; thus, the remaining embryos were aborted later (Agrawal, Bharadwaj, and Mathur 1980).

A second experiment evaluating the abortifacient activity of Juniperus Communis Extract was conducted according to the procedure in the preceding paragraph, with the exception that doses (300 or 500 mg/kg) were administered to groups of five rats only on days 14, 15, and 16 of pregnancy. Following parturition, the number of litters delivered was determined. In both dose groups, early or late abortions resulted and no pups were born. All control rats had implantation sites on day 18, and the average number of pups delivered was 9.5 $\pm$ 1.8. Neither body weight loss nor side effects was/were noted in this experiment or the preceding experiment (Agrawal, Bharadwaj, and Mathur 1980).

In a third set of experiments, three of the rats without implants on day 10 were allowed to mate with males after 2 months of

rest. Although the matings were successful, no implantations were reported (Agrawal, Bharadwaj, and Mathur 1980).

Based on the results of the preceding three experiments, the investigators concluded that Juniperus Communis Extract had antifertility and abortifacient effects in rats, but was not teratogenic.

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation

Juniperus Communis Oil

Two irritation reactions were observed in 2 of 20 subjects patch-tested with Juniper Berry Oil (full strength) for 24 hours (Research Institute for Fragrance Materials 1976). Juniper Berry Oil (8% in petrolatum) did not cause skin irritation in human subjects patch-tested for 48 hours (closed patches) (Research Institute for Fragrance Materials 1976).

Juniperus Virginiana Oil

The skin irritation potential of 0.2%, 2.0%, and 20.0% Juniperus Virginiana Oil (Cedarwood Oil, Virginia) was evaluated using male and female subjects. The groups tested (24–72-hour closed patch test) consisted of normal subjects and those with dermatoses. Skin irritation was not observed in any of the following groups: 0.2% Juniperus Virginiana Oil (148 subjects), 2.0% (30 subjects), and 20.0% (29 subjects) (Fujii, Furukawa, and Suzuki 1972).

Irritation was not observed in the skin of the backs of five male subjects patch tested (48-hour closed patch test) with 8% Juniperus Virginiana Oil in petrolatum (Research Institute for Fragrance Materials 1974).

Juniperus Oxycedrus Tar

Juniper Tar (2% in petrolatum) did not induce skin irritation in subjects patch tested for 48 hours in a closed patch test (Research Institute for Fragrance Materials 1975).

Skin Irritation/Sensitization

Juniperus Communis Oil

The sensitization potential of Juniper Berry Oil (8% in petrolatum) was evaluated in the maximization test (Kligman 1966; Kligman and Epstein 1975) using 25 volunteers. No evidence of sensitization was observed in any of the subjects tested (Research Institute for Fragrance Materials 1976).

In a more recent study, 86 of 299 patients with allergic reactions to an International Contact Dermatitis Research Group (ICDRG) perfume mixture containing one essential oil and seven other fragrance substances were tested with Juniper Berry Oil and 34 other essential oils according to the procedure of Rudzki, Grzywa, and Brud (1976). Six of the 86 subjects were sensitive to Juniper Berry Oil (Rudzki and Grzywa 1986).

Juniperus Virginiana Oil

The incidence of sensitization to Juniperus Virginiana Oil (a.k.a. Cedarwood Oil, Virginia; 10% in petrolatum) was evaluated using 20 children (1–13 years old), 16 of whom had dermatitis. Patch tests were applied to the upper back, and reactions were scored according to ICDRG criteria at 30 minutes and 2, 4, and 10 days. The following grading scale was used: − (negative reaction) to + + + (bullous reaction). Sensitization reactions were not observed (Abifadel et al. 1992).

In another study, the sensitization potential of 8% Juniperus Virginiana Oil in petrolatum was evaluated using the maximization test procedure (Kligman 1966). No evidence of sensitization was observed in any of the 25 male subjects tested (Research Institute for Fragrance Materials 1974).

Eighty-one contact dermatitis patients were patch tested (closed patch tests) with 5% Juniperus Virginiana Oil in white petrolatum. One sensitization reaction was observed at 24 hours and another at one week (Ishihara 1977).

The incidence of sensitization to Juniperus Virginiana Oil was evaluated in 95 patients using 2-day patch tests (closed patches). Petrolatum served as the vehicle for the test substance. Reactions were scored according to ICDRG criteria on days 2 and 3 or days 2 and 4. None of the patients had irritation or sensitization reactions to 1% or 5% Juniperus Virginiana Oil in petrolatum (Frosch et al. 1995).

Sensitization reactions were observed in 6 of 450 dermatitis patients tested with 2% Juniperus Virginiana Oil in yellow, soft paraffin. Details concerning the experimental procedure were not included (Rudzki and Grzywa 1977).

Juniperus Oxycedrus Tar

Weakly positive patch test reactions (irritation) were observed in 43 of 242 patients patch tested with 10% Juniper Tar in vaseline (Van Andel, Bleumink, and Nater 1974).

The sensitization potential of Juniper Tar (2% in petrolatum) was evaluated using 25 subjects according to maximization test procedures (Kligman 1966; Kligman and Epstein 1975). Sensitization reactions were not observed in any of the subjects tested (Research Institute for Fragrance Materials 1975).

A total of 650 patients with skin disorders resembling contact dermatitis were patch tested with a series of common contact allergens. Patch tests were conducted according to the methods of the ICDRG. Patches were applied to the back and removed after 48 hours. Reactions were scored at 48 and 72 hours postapplication. Thirty-three of the 59 patients with positive reactions to a wood tar mixture containing 3% Juniper Tar were selected for further testing. Of the 33 patients, 20 had positive reactions to the wood tar mixture when retested. When these 20 patients were patch tested with Juniper Tar (0.010–0.015 ml of 3% solution in acetone), the following reactions were observed in 13 subjects: + (5 subjects), 2+ (5 subjects), and 3+ (3 subjects). Irritant reactions were not observed (Van Andel, Bleumink, and Nater 1974).

Phototoxicity

Juniperus Communis Extract

Juniper Extract (undiluted leaf extract) was applied to three sites on the dorsal skin of 25 volunteers using aluminum cups ("Finn-Chambers") fixed to microporous tape. Photopatch tests were performed on two sites; the third site served as the control. Sites were irradiated with a sun simulator (OSRAM lamp, 2500 W). The first site was irradiated with UVA light (315–400 nm; dose = 10 J/cm^2), and the second site was irradiated with UVB light (280–315 nm). Exposure at the UVB irradiated site was 75% of the Minimal Erythema Dose (MED). The MED was determined after irradiation of small areas at increasing doses according to the Saidman test protocol. Reactions were scored after 48 and 72 h according to the following scale: + (erythema) to ++++ (bullae). Reactions were interpreted according to the following guidelines: positive reactions to three tests (patch test, photopatch test UVA + photopatch test UVB) indicate irritant activity for a given product. A clearly increased reaction to photopatch tests compared to control epicutaneous reaction indicates photoaggravation. A positive reaction to photopatch tests (either UVB or UVA, but, in practice, mainly UVA) indicates phototoxicity, subject to negativity of reference patch test. Juniper Extract did not induce irritation, photoaggravation, or phototoxicity (Bouhlal et al. 1989).

The leaf extract of Juniperus Communis is not reviewed in this report. However, the preceding negative phototoxicity data are included because of the similar finding of negative results for Juniperus Communis Oil (from berries) in hairless mice and swine included earlier in this report. Different parts of the same juniper plant (leaves and berries) could be similar in their potential for inducing phototoxicity. Thus, the human phototoxicity data on Juniperus Communis leaf extract could prove to be useful in evaluating the phototoxicity potential of Juniperus Communis Extract (from berries) in humans, in the absence of data on the latter ingredient.

Case Reports

A 53-year-old aromatherapist with an acute bilateral hand eczema was patch tested with the European standard series (Trolab) and to working dilutions of essential oils. Juniper Oil (1% in grapeseed oil) did not induce any reactions at 48 or 96 hours (Bilsland and Strong 1990).

A 47-year-old patient with eczema of the face and hands had been exposed to a spice (used in production of sausage) containing Juniper Berry Oil and a smoking powder (used in smoking sausage) containing juniper over a period of 25 years. The patient was a sausage vendor and had to enter a smokehouse repeatedly during the transportation of meat and sausage products. Difficulty in breathing, wheezing, and coughing (asthma symptoms) were noted, particularly on days in which sausage was smoked. The patient also had papular reactions to Juniper Oil in an epidermal test. Intracutaneous test results indicated a + reaction to Juniper Oil in Polysorbate 20 (1:5,000) and a + reaction to Juniper Oil in Polysorbate 20 (1:500). Additionally, results of an inhalational allergen test (IAT) indicated an unequivocal obstructive reaction 15 minutes after inhalation of Juniper Oil in Polysorbate 20 (1:100), the highest concentration tested. Papules were observed on the hands 24 hours after the initial IAT. No pathological findings were identified in pulmonary function tests. The results of each test performed collectively indicated that the patient's contact dermatitis and bronchial asthma were due to occupational exposure to Juniper Oil. No further reports of skin reactions or respiratory problems were observed after the patient began working in an office (Rothe, Heine, and Rebohle 1973).

A 54-year-old woman with intense, acute eczema was patch-tested with Juniperus Virginiana Oil (Finn chambers) in petrolatum. The eczema resulted from dermal use of a mixture of incense and brandy. Sensitization was noted at a test concentration of 10% in petrolatum on day 2, but not at 20 minutes or on day 4 (Basto and Azenha 1991).

SUMMARY

This safety assessment is on the following ingredients that are listed in the International Cosmetic Ingredient Dictionary: Juniperus Communis Extract (fruit extract), Juniperus Oxycedrus Extract (fruit extract), Juniperus Oxycedrus Tar (from wood), Juniperus Phoenicea Extract (gum extract), and Juniperus Virginiana Extract (wood extract). Common juniper, from which various extracts and tars are derived, is a short to medium height tree that grows wild in many parts of Europe, Asia, and North America.

Because similarities regarding the composition of Juniper Berry Oil and Juniper Extract (fruit extract) have been identified in the published literature (e.g., Juniper Berry Oil and Juniper Extract, from *J. communis* L. ssp. *nana Syme*, have the same qualitative composition), data on Juniperus Communis Oil are included in this report for use in the safety assessment of Juniperus Communis Extract. Similarly, data on Juniperus Virginiana Oil will be included for use in the safety assessment of Juniperus Virginiana Extract. The Oils of juniper and other forest trees can be very complex, containing hundreds of terpenoids and aromatic compounds. Occasionally, important amounts of aliphatic alcohols and aldehydes, and more rarely, alkanes are also present.

Juniperus Communis Extract, Juniperus Oxycedrus Extract, Juniperus Phoenicea Extract, and Juniperus Virginiana Extract function as biological additives in cosmetics. Juniperus Oxycedrus Tar is used as a hair conditioning agent and as a fragrance component in cosmetics.

Product formulation data submitted to the FDA in 1998 indicated the following use frequencies for Juniper Extract, Juniper Tar, and related ingredients: Juniper Extract (47 products), Juniper Berry Oil (9 products), Juniper Oil (4 products), and Juniper Tar (8 products).

The acute oral LD_{50} for Juniper Berry Oil and Juniperus Virginiana Oil was >5 g/kg in two different studies. An acute

oral LD_{50} of 8.0 g/kg (rats) was reported for Juniperus Oxycedrus Tar.

In two studies involving rabbits, the acute dermal LD_{50} for Juniperus Communis Oil and Juniperus Virginiana Oil was >5g/kg. The acute dermal LD_{50} for Juniper Tar in rabbits was also >5 g/kg.

The intraperitoneal administration of 10% Juniper Oil (juniper species not stated) did not impair coordination of movement or induce muscle relaxation in mice or guinea pigs. The same was true for mice dosed intramuscularly and guinea pigs dosed subcutaneously with the test solution. When the same test solution was administered to rabbits (oral and intramuscular administration) in doses 10× that considered therapeutic (therapeutic dose = 2–3 g/kg), no pronounced toxic reactions were detected.

Undiluted Juniperus Communis Oil did not induce skin irritation when applied to the backs of hairless mice and swine. Moderate skin irritation was observed after the undiluted oil was applied to intact or abraded skin of rabbits.

Juniperus Virginiana Oil also did not induce skin irritation when applied to the backs of hairless mice. Furthermore, this ingredient induced neither skin irritation nor systemic toxicity when applied to the backs of 101 inbred mice. The two applications were separated by a 7-day interval. Moderate skin irritation was observed after undiluted Juniperus Virginiana Oil was applied (occlusive patches) to the skin of rabbits for 24 hours.

Skin irritation was not observed after undiluted Juniperus Oxycedrus Tar was applied to the backs of hairless mice, or following application to intact or abraded skin of rabbits for 24 hours.

In an open, epicutaneous test involving groups of six to eight guinea pigs, 8% Juniperus Virginiana Oil was not a sensitizer.

Undiluted Juniperus Communis Oil was not phototoxic when applied to hairless mice and swine. Undiluted Juniperus Virginiana Oil also was not phototoxic when applied to the backs (20 μl per 2 cm^2) of six hairless mice and two miniature swine. Similarly, negative results for Juniper Tar were reported in a phototoxicity study involving hairless mice and swine.

Juniperus Oxycedrus Tar was classified as a weak agent in a bacterial (*B. subtilis*) assay for DNA damage, the rec assay. This tar was also mutagenic in reverse mutation assays using *S. typhimurium* or *E. coli* tester strains.

Aromatic DNA adducts were induced in human and mouse skin (in vivo) and human skin (in vitro) treated with Juniperus Oxycedrus Tar. In consideration of the extent of DNA adduct formation in these tests and the absence of carcinogenicity data on Juniperus Oxycedrus Tar, the investigators stated that it is expected that this ingredient would be active on mouse skin. The investigators in two other experiments (in vitro and in vivo) involving human skin samples and another experiment involving mouse skin (in vivo) stated that the results provide direct evidence for the formation of potentially carcinogenic DNA damage in human and mouse tissue by Juniper Tar and other components of the therapeutic tar preparations.

In albino rats dosed orally, Juniperus Communis Extract induced antifertility and abortifacient effects, but had no teratogenic effects.

Skin irritation was observed in 2 of 20 subjects patch tested (24-hour application) with Juniperus Communis Oil. Juniperus Communis Oil (8% in petrolatum) did not induce skin irritation in subjects patch tested for 48 hours (closed patches).

Skin irritation was not observed in either of the following three groups patch tested (24–72-hour closed patch tests) with different concentrations of Juniperus Virginiana Oil: 0.2% Juniperus Virginiana Oil (148 subjects), 2.0% (30 subjects), and 20.0% (29 subjects). Also, at a concentration of 8% in petrolatum (48-hour closed patch test), Juniperus Virginiana Oil did not induce skin irritation in any of the five subjects tested.

Juniper Tar (2% in petrolatum) did not induce skin irritation in either of the subjects participating in a 48-hour closed patch test.

In the maximization test, no evidence of sensitization was observed in any of the 25 subjects tested with 8% Juniperus Communis Oil in petrolatum. In another study in which 86 of 299 patients with allergic reactions to a perfume mixture were tested with various essential oils, 6 of the 86 were sensitive to Juniperus Communis Oil.

No evidence of sensitization was observed in 20 children (16 were dermatitis patients) patch tested with 10% Juniperus Virginiana Oil in petrolatum. Also, no evidence of sensitization was observed in 25 subjects tested with 8% Juniperus Virginiana Oil in petrolatum in the maximization test.

Sensitization was observed in 1 of 81 contact dermatitis patients patch tested (closed patch tests) with 5% Juniperus Virginiana Oil in white petrolatum. However, neither irritation nor sensitization was observed in 95 patients patch tested (closed patches) with 1% or 5% Juniperus Virginiana Oil in petrolatum.

Six of 450 dermatitis patients had sensitization reactions to 2% Juniperus Virginiana Oil in yellow, soft paraffin.

Weakly positive patch test reactions (irritation) were observed in 43 of 242 patients patch tested with 10% Juniper Tar in vaseline. Juniper Tar (2% in petrolatum) did not induce sensitization in any of the 25 subjects patch tested in the maximization test.

Thirty-three of 59 patients with positive reactions to a wood tar mixture containing 3% Juniper Tar were selected for further patch testing (48-hour patch test) with the same mixture. Reactions were observed in 20 of the 33 subjects. Subsequent patch testing of the 20 subjects with 3% Juniper Tar in acetone yielded the following results: + reaction (5 subjects), 2+ (5 subjects), and 3+ (3 subjects). Irritant reactions were not observed.

Juniperus Communis Extract (undiluted leaf extract) did not induce irritation, photoaggravation, nor phototoxicity in 25 volunteers. The leaf extract of Juniperus Communis is not being reviewed in this safety assessment; however, these results are

included in light of similar results for Juniperus Communis Oil (from berries) in hairless mice and swine.

DISCUSSION

Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be enough to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on Juniperus Communis Extract, Juniperus Oxycedrus Extract, Juniperus Oxycedrus Tar, Juniperus Phoenicea Extract, and Juniperus Virginiana Extract were insufficient to determine whether these ingredients, for purposes of cosmetic use, are either safe or unsafe. The Expert Panel released a Notice of Insufficient Data Announcement on June 6, 1997, outlining the data needed to assess the safety of these ingredients.

The types of data required include[2]

1. Current concentration of use data on each Juniper Extract and Tar and function in cosmetics.
2. Methods of manufacture and impurities data, particularly relating to the presence of pesticide residues, on each Juniper Extract and Tar.
3. UV absorption spectra on each Juniper Extract and Tar; if there is significant absorbance in the UVA or UVB range, then a human photosensitization study on all ingredients may be needed.
4. Developmental and reproductive toxicity study to include determination of the no-effect level on implantation of each Juniper Extract and Tar.
5. Two different genotoxicity assays on each Juniper Extract (one using mammalian system); if positive, a dermal carcinogenicity assay by National Toxicology Program (NTP) standards will be requested for each ingredient.
6. A dermal carcinogenicity assay by NTP methods on Juniper Tar.
7. Irritation and sensitization data (animal or human) on each Juniper Extract and Tar.

No offer to supply the data was received.

On December 9, 1997, the CIR Expert Panel reached a tentative conclusion that the available data were insufficient to support the safety of these ingredients (citing the above data needs) and issued a Tentative Report for comment. No comments or additional data were received in the 90-day public comment period provided.

Therefore, in accordance with Section 45 of the CIR Procedures, the Expert Panel has issued a Final Report with an Insufficient Data conclusion. When the requested data are available, the Expert Panel will consider the Final Report in accordance with Section 46 of the CIR Procedures, Amendment of a Final Report.

[2]All testing should be done on cosmetic grade ingredient.

CONCLUSION

The Expert Panel concludes that the available data are insufficient to support the safety of Juniperus Communis Extract, Juniperus Oxycedrus Extract, Juniperus Oxycedrus Tar, Juniperus Phoenicea Extract, and Juniperus Virginiana Extract for use in cosmetic products.

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