ISOEUGENOL
CAS Number 97-54-1

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Submitted to:
NATIONAL TOXICOLOGY PROGRAM

Submitted by:
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Board of Scientific Counselors Draft Report

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OVERVIEW

Nomination History: Isoeugenol was originally nominated for carcinogenicity testing by the National Cancer Institute (NCI) in 1979. However, because of budgetary cutbacks it was recommended for genetic toxicology screening. In 1984, NCI renominated isoeugenol for carcinogenicity testing with low to moderate priority based on its structural similarity to the carcinogens eugenol, safrole, isosafrole, and estragole, and its potential for human exposure as a food flavoring agent and a fragrance ingredient.

Chemical and Physical Properties: Isoeugenol is an oily yellowish compound that occurs in a liquid (cis) or crystalline (trans) state. The melting point of isoeugenol is 33°C (91.0°F) (trans). The boiling point has been reported to be 133.0°C (271.4°F) (cis) and 140.0°C (284.0°F) (trans). Isoeugenol is slightly soluble in water.

Production/Uses/Exposure: Isoeugenol is produced by various companies throughout the United States and Europe. The production volume of isoeugenol by 5 manufacturers was reported in the public file of the EPA Toxic Substances Control Act (TSCA) Inventory in 1983 to range from of 21,000-212,000 pounds. Isoeugenol is used to manufacture vanillin, and is widely used in fragrances and as a flavoring additive. Many consumers are potentially exposed to isoeugenol from its use in cosmetics and food. Data from the National Occupational Exposure Survey (NOES), conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimate that 35,167 workers, including 24,978 female employees, were potentially exposed to isoeugenol in the workplace. OSHA has not established a PEL for isoeugenol. ACGIH has not recommended a TLV and NIOSH has not recommended a REL for this compound.

Toxicological Effects:

Human: Isoeugenol has been shown to cause contact and allergic dermatitis in humans. Positive skin patch tests in numerous individuals have confirmed the sensitizing ability of isoeugenol. There were no data found on chemical disposition, chronic, carcinogenic, reproductive, or teratogenic effects of isoeugenol in humans.

Animal: Isoeugenol was found to induce low to severe irritation reactions when applied to animal skin. Rats and rabbits were found to be extremely sensitive to isoeugenol following its topical application, whereas swine were less sensitive. Isoeugenol has been found to cause skin sensitization in guinea pigs. The LD₅₀ of isoeugenol was determined to be 1560 mg/kg, 1410 mg/kg, and 316 mg/kg for rats, guinea pigs, and birds, respectively. There were no data found on chemical disposition, chronic, carcinogenic, reproductive, or teratogenic effects of isoeugenol in animals.

Genetic Toxicology:

Humans: Isoeugenol was found to increase significantly (P<0.01) sister-chromatid exchanges in cultured human lymphocytes. Isoeugenol was non-mutagenic to Salmonella typhimurium and Escherichia coli, with and without metabolic activation. The chemical was also non-mutagenic to Saccharomyces cerevisiae and cultured Chinese hamster ovary cells.

Structure Activity Relationships: Isoeugenol is structurally related to eugenol, for which there is equivocal
evidence of carcinogenicity in mice. Isoeugenol is also structurally related to safrole and isosafrole which have been found to be carcinogenic in mice and rats, estragole which has been found to be carcinogenic in mice, and methyleugenol, which has induced pathologic effects in rats and mice in a subchronic toxicity study.

1 The information contained in this Executive Summary of Safety and Toxicity Information (ESSTI) is based on data from current published literature. The summary represents information provided in selected sources and is not claimed to be exhaustive.
I. NOMINATION HISTORY AND REVIEW

A. Nomination History

1. Source: National Cancer Institute (NCI) [NCI, 1984a, b]

2. Date: October, 1984

3. Recommendations:
   
   • Carcinogenicity

4. Priority: Low-to-Moderate

5. Rationale/Remarks:
   
   • Previous recommendation (1979) for carcinogenicity but due to budgetary cutbacks in 1982 it was recommended for genetic toxicology screening. Methyleugenol selected to represent the alkoxyphenol ethers in an in-depth toxicological evaluation.
   
   • NCI renominated isoeugenol (1984) based on the following considerations:
     
     ○ Structurally similar to eugenol, for which there is equivocal evidence of carcinogenicity in mice, and to the carcinogens safrole, isosafrole, and estragole.
     
     ○ Concern about the reactivity of the propenyl moiety. Interest in determining whether the closer proximity of the double bond to the aromatic ring (as compared to that in methyleugenol) would better facilitate epoxidation.
     
     ○ Potential for human exposure as a food flavoring agent and fragrance ingredient.
     
     ○ NTP prechronic studies of methyleugenol completed (review for further evaluation in progress).

B. Chemical Evaluation Committee Review

1. Date of Review: March 13, 1991

2. Recommendation:
   
   • Chemical disposition
   • Reproductive and developmental effects
   • Carcinogenicity

3. Priority: Low to moderate

4. NTP Chemical Selection Principles: 3, 8

5. Rationale/Remarks:
   
   • High occupational and consumer exposure
   • Relatively high female industrial exposure
   • Widespread use in fragrances and as a flavoring agent
   • Naturally occurring product
   • Evidence of genotoxicity in human cells
   • Structurally related to compounds with known or suspect carcinogenic potential
C. Board of Scientific Counselors Review

1. Date of Review:

2. Recommendations:

3. Priority:

4. Rationale/Remarks:

D. Executive Committee Review

1. Date of Review:

2. Decision:
II. CHEMICAL AND PHYSICAL DATA

A. Chemical Identifiers

ISOEUGENOL

Molecular Formula: $C_{10}H_{12}O_2$  
Molecular Weight: 164.22

$CAS \text{ No. 97-54-1}$

$RTECS \text{ No. SL7875000}$

B. Synonyms and Tradenames

**Synonyms:** phenol 2-methoxy-4-propenyl (8CI); phenol, 2-methoxy-4-(1-propenyl)(9CI); 4-hydroxy-3-methoxy-1-propenylbenzene; 2-methoxy-4-(1-propenyl) phenol; 2-methoxy-4-propenylphenol; 4-propenylguaiacol

**Trade names:** No data were available.

C. Chemical and Physical Properties

**Description:** Oily liquid which turns yellow easily. Exists as liquid ($cis$) or crystals ($trans$) [Budavari et al., 1989] and has a spice clove type odor [BRS, 1990]

**Melting Point:**
- 33.0°C (91.0°F) ($trans$) [Budavari et al., 1989]
- -10°C (-50°F) ($mixture$) [Budavari et al., 1989]

**Boiling Point:**
- 133.0°C (271.4°F) at 11 mm Hg ($cis$) [Budavari et al., 1989]
- 140.0°C (284.0°F) at 12 mm Hg($trans$) [Budavari et al., 1989]
- 128-130°C (262.4-266°F) ($mixture$) [Budavari et al., 1989]

**Specific Gravity:**
- 1.077 [Aldrich, 1990]
1.080 [Budavari et al., 1989]
1.0837 (cis) [Weast, 1989]
1.088 (cis) [Budavari et al., 1989]
1.0852 (trans) [Weast, 1989]
1.087 (trans) [Budavari et al., 1989]

Refractive Index:

- 1.5760 [Aldrich, 1990]
- 1.5778 (trans) [Budavari et al., 1989]
- 1.5724 (cis) [Budavari et al., 1989]
- 1.5739 (mixture) [Budavari et al., 1989]

Solubility in water: Slightly soluble in water [Budavari et al., 1989] (0.0002%-0.0005%) [Jimbo et al., 1983]

Solubility in Other Solvents: Miscible in alcohol, ether [Budavari et al., 1989] ethanol and propylene glycol [Jimbo et al., 1983]

Log Octanol/Water Partition Coefficient: No data were found.

Reactive Chemical Hazards: Decomposition products include acrid smoke and fumes [Sax and Lewis, 1989].

Flammability Hazards:

- Combustible [NFPA, 1986]
- Flash point: >110°C (230°F) CC [Aldrich, 1990]; >100°C(212°F) CC [NFPA, 1986]
- Vapor Density: No data were found.
- Autoignition Temperature: No data were found.
- Flammable Limits in Air: No data were found.
III. PRODUCTION/USE

A. Production

1. Manufacturing Process

Isoeugenol is prepared by isomerization of eugenol with caustic potash [Sax and Lewis, 1987]. Eugenol is found in the volatile oils from clove, pimenta, bay leaves, Ceylon cinnamon, camphor, sassafras, canella and other oils [Gennaro, 1985]. It is principally obtained from clove oil which contains 80% eugenol [Gosselin et al., 1984]. Clove oil is treated with excess sodium hydroxide solution to dissolve the eugenol. The resulting mixture is then extracted with ether to remove the other constituents in the oil, leaving behind an aqueous solution of sodium eugenol. The aqueous solution is then acidified to yield eugenol, which is then purified by distillation [Gennaro, 1985].

2. Producers and Importers

U.S. Producers
Berje, Inc.
Bloomfield, New Jersey
Biddle Sawyer, Inc.
Keyport, New Jersey
Chem-Fleur, Inc.
Newark, New Jersey
Chemical Division-UOP, Inc.
East Rutherford, New Jersey
Elan Chemical Company, Inc.
Newark, New Jersey
Firmenich, Inc.
Princeton, New Jersey
Fritzsche Dodge and Olcott, Inc.
East Hanover, New Jersey
Givaudan Corporation
Clifton, New Jersey
Haarmann and Reimer Corporation
Springfield, New Jersey
Norda, Inc.
Boonton, New Jersey
Penta Manufacturing Company
Fairfield, New Jersey
Schweizerhall, Inc. Chemical Division
South Plainfield, New Jersey
Unilever United States, Inc. Quest International
Boonton, New Jersey

European Producers
Bush Boake Allen LTD
Sudbury, United Kingdom

Reference
Chemical Week Buyers' Guide, 1990;
DCI, 1990
USEPA, 1990
SRI, 1990;
Chemical Week Buyers' Guide, 1990;
DCI, 1990
USEPA, 1990
USEPA, 1990
Chemical Week Buyers' Guide, 1990;
DCI, 1990
USEPA, 1990
Chemical Week Buyers' Guide, 1990;
DCI, 1990
USEPA, 1990
Chemical Week Buyers' Guide, 1990;
DCI, 1990
Chemical Week Buyers' Guide, 1990
USEPA, 1990
SRI, 1990;
Chemical Week Buyers' Guide, 1990;
DCI, 1990
Chemical Week Buyers' Guide, 1990
SRI, 1990
SRI, 1990
Charabot SA  SRI, 1990
Grasse, France

Givaudan France  SRI, 1990
Lyon, France

Haarmann and Reimer GmbH  SRI, 1990
Holzminden, Germany

Lautier SA-Florasynth  SRI, 1990
Grasse, France

Quest International U.K. Ltd.  SRI, 1990
Ashford, United Kingdom

V. Mane Fils SA  SRI, 1990
Le Bar Sur Loup, France

**Importers**

EM Laboratories, Inc  USEPA, 1990
Elmsford, New York

Haarmann and Reimer GmbH  USEPA, 1990
Springfield, New Jersey

Polak's Frutal Works, Inc.-NY  USEPA, 1990
Middletown, New York

Polarome Manufacturing  USEPA, 1990
New York, New York

Roure Bertrand DuPont Inc.  USEPA, 1990
Teaneck, New Jersey

Synarome Corporation  USEPA, 1990
New York, New York

Ungerer and Company  USEPA, 1990
Totowa, New Jersey

V. Mane Fils, Inc.  USEPA, 1990
Fairfield, New Jersey

3. Volume

The production volume of isoeugenol is reported in the public file of the EPA Toxic Substances Control Act (TSCA) Inventory. In 1983, 9 manufacturers were listed as producers of isoeugenol. Five reported a total production volume ranging from 21,000-212,000 pounds. Four additional manufacturers did not report production information [USEPA, 1990].

Isoeugenol is listed in the United States International Trade Commission's Publication *Synthetic Organic Chemicals*. However, no production data were available on isoeugenol from this source for the years 1985-1989 *[USITC, 1986-1990]*.

It is estimated that 175,000 pounds of isoeugenol are utilized annually in the United States in the production of fragrances [Thompson, et al., 1983].

The import volume of isoeugenol is reported in the public file of the EPA TSCA Inventory. In 1983, 8 companies were listed as importers of isoeugenol. 4 companies listed as importers reported a total import volume ranging from 12,000-122,000 pounds. Four additional importers did not report volume information [USEPA, 1990].
The volume of imported eugenol/isoeugenol has almost doubled every year since 1986 [Chemical Marketing Reporter, 1989]. The combined net quantities of isoeugenol and eugenol exported to the United States between 1985 and 1988 are reported in Table 1 [USDC, 1986-1989].

4. Technical Product Composition

Isoeugenol is available as a mixture of cis and trans isomers at a purity of 99% [Aldrich, 1990].

B. Use

- Flavoring agent [Sax and Lewis, 1987] for non-alcoholic beverages, baked goods, and chewing gum [Wisneski et al., 1988].
- Manufacture of vanillin [Budavari et al., 1989].
- Fragrance ingredients in perfumes (0.4%-0.8%) [Wisneski et al., 1988; Opdyke, 1975], soaps (0.03%-0.3%), detergent (0.003%-0.3%), and cream lotions (0.015%-0.1%). Use as a fragrance ingredient in the United States amounts to approximately 40,000 pounds/year [Opdyke, 1975].

The concentration of isoeugenol in various foods and beverages is presented in Table 2 [Furia and Bellanca, 1975].

* Production statistics for an individual chemical are given only when there are three or more producers, no one or two of which may be predominant. Moreover, even when there are three or more producers, statistics are not given if there is any possibility that their publication would violate the statutory provisions relating to unlawful disclosure of information accepted in confidence by the Commission. Data are reported by producers for only those items where the volume of production or sales or value of sales exceeds certain minimums. Those minimums for all sections are 5,000 pounds of production or sales or $5,000 of value of sales with the following exceptions: plastics and resin materials-50,000 pounds or $50,000; pigments, medicinal chemical, flavor and perfume materials, and rubber processing chemicals-1,000 pounds or $1,000.
IV. EXPOSURE/REGULATORY STATUS

A. Consumer Exposure

Since isoeugenol is used in a variety of consumer products, including beverages, baked goods, and perfume, there is potential for consumer exposure.

B. Occupational Exposure

Data from the National Occupational Exposure Survey (NOES), conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimate that 35,167 workers, including 24,978 female employees, were potentially exposed to isoeugenol in the workplace. The NOES data base does not contain information on the frequency, level or duration of exposure of workers to any chemicals listed therein [NIOSH, 1990].

C. Environmental Occurrence

Isoeugenol occurs naturally in Ylang-Ylang, clove, tuberose, jonquil, nutmeg [Kirk-Othmer, 1983; Opdyke, 1975], tobacco [Wynder and Hoffmann, 1967], and sandalwood [Demole et al., 1976]. Isoeugenol has also been identified in dill seed, mace, Jamaican rum, smoked pork belly [Kirk-Othmer, 1983], gardenia [Hattori, et al., 1978], and other flowers [NCI, 1984b].

Isoeugenol has been found to be one of approximately 300 constituents identified in pulp and paper mill effluents. However, no studies have been performed to determine its impact on soil, water, air, or vegetation [Nestmann et al., 1980].

D. Regulatory Status

- Isoeugenol is approved for food use by the Food and Drug Administration when used in the minimum quantity required to produce its intended effect, and otherwise in accordance with all the principles of good manufacturing practices {21 CFR 172.515} [Office of the Federal Register, 1990].
- The Food and Drug Administration (FDA) has classified isoeugenol as GRAS (Generally Recognized As Safe) when used in moderate amounts per FDA specifications {21 CFR 172.515} [Office of the Federal Register, 1990].
- OSHA has not established a permissible exposure limit (PEL) for isoeugenol.

E. Exposure Recommendations

- ACGIH has not recommended a threshold limit value (TLV) for isoeugenol.
- NIOSH has not recommended an exposure limit (REL) for isoeugenol.
V. TOXICOLOGICAL EFFECTS

A. Chemical Disposition

1. Human Data

dermal, human

- The penetration of isoeugenol through human epidermis has been studied. The lower abdominal epidermal skin from human cadavers was dosed with 10 mM carbon-14 labelled isoeugenol (specific activity of 10 mCi/ml) in various test vehicles for 24 hours. The vehicles tested were 5 liquid vehicles (ethanol, ethanol/water (85:15 v/v), ethanol/water (70:30 v/v), propylene glycol, and liquid paraffin), 6 cosmetic vehicles (lotion, milky lotion, o/w-type cream, w/o-type cream, o/w-type foundation, and oil type foundation) (See Tables 3-8 [Table 3. Lotion Composition, Table 4. Milky Lotion Composition, Table 5. O/W type Cream Composition, Table 6 W/O Type Cream Composition, Table 7. O/W type Foundation Composition, Table 8. Oil type Foundation Composition] for the chemical composition for each of these vehicles); and 2 ointment vehicles (white petrolatum and macrogol ointment). For the liquid vehicles, 0.2 ml of each sample was applied. For the 5 cosmetic and 2 ointment vehicles, the test sample volume was 0.2 cm³. The percentage of penetration in liquid vehicles ranged from 0.29 ±0.04% (ethanol) to 4.31 ± 0.84% (liquid paraffin). The percent penetration was significantly greater (P<0.01) from liquid paraffin compared to the other 4 vehicles. The penetration of each compound showed a tendency to increase in direct proportion to the ratio of water to ethanol.

The percentage of penetration in cosmetic and ointment vehicles ranged from 0.05 ± 0.01% (macrogol ointment) to 10.38 ± 0.63% (milky lotion). The percent penetration of isoeugenol from milky lotion was significantly (P<0.01) greater than from the other 7 cosmetic/ointment vehicles. The authors reported that the increased penetration afforded by this vehicle may be due to the water content of milky lotion [Jimbo et al., 1983].

2. Animal Data

No data were found on the chemical disposition of isoeugenol in animals.

B. Acute

1. Human Data

dermal, human

- Fifty adult male volunteers with no known allergies were subjected to dermal patch testing with isoeugenol in order to investigate the irritation potency of this compound. Lint patches with 0.05 grams of 32% isoeugenol in acetone were placed on the skin of the volunteers' backs for 48 hours. Based on readings at 48, 72, 96 and 120 hours, the irritation potential of isoeugenol was determined to be moderate [Motoyoshi et al., 1979].

2. Animal Data

oral, rats

- The acute LD₅₀ of isoeugenol was determined using groups of 10 (5 male, 5 female per dose level) adult Osborne-Mendel rats. Unspecified doses of neat isoeugenol were administered to the rats by
stomach tube after 18 hours of fasting. The LD$_{50}$ for isoeugenol was determined to be 1560 (range 1290-1880) mg/kg. Prior to death, the rats appeared scrawny after one dose and became comatose. Death occurred within 1 hour to 7 days of administration [Jenner et al., 1964].

- A group of 6 (3 male, 3 female) adult Osborne-Mendel or Sherman rats was used to determine the acute hepatotoxicity of isoeugenol. The rats were administered a dose of 520 mg/kg (1/3 the LD$_{50}$) via stomach tube daily for 4 days. On the fifth day, 5 of the 6 rats (one died prior to the fifth day) were sacrificed to determine whether any macroscopic liver lesions occurred. No macroscopic liver lesions were observed in the rats [Taylor et al., 1964].

**oral, guinea pigs**

- The acute oral LD$_{50}$ of isoeugenol for an unspecified strain and number of male and female guinea pigs was determined. Guinea pigs were given unspecified doses of isoeugenol by intubation after 18 hours of fasting. The LD$_{50}$ for isoeugenol was determined to be 1410 (range 1130-1780) mg/kg. Prior to death, the guinea pigs were observed to be depressed and became comatose. Death occurred within 3 to 6 days [Jenner et al., 1964].

**oral, birds**

- The acute oral LD$_{50}$ in quail, and the repellency-toxicity index (R$_{50}$), which is analogous to an LD$_{50}$ for redwing blackbirds, was determined for isoeugenol. An unspecified amount of isoeugenol suspended in propylene glycol was administered to an unspecified number of birds (redwing blackbirds and coturnix quail) via gavage. The LD$_{50}$ for isoeugenol in quail was determined to be greater than 316 mg/kg. The R$_{50}$ for redwing blackbirds was found to be greater than 1.00 percent [Schafer et al., 1983].

**dermal, guinea pigs**

- Ten female Hartley albino guinea pigs were used to study the irritant potential of isoeugenol. The flank areas of the guinea pigs were shaved, and a dose of 20 mg of 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0% isoeugenol in petrolatum was applied (in Finn chambers on Scanpor tape) for 48 hours. The degree of reaction was evaluated at 1, 24, and 48 hours after the isoeugenol was removed. The results indicated that isoeugenol produced an irritant response at concentrations of 5.0 and 10.0% only [Itoh, 1982].

- A group of 6 male Hartley guinea pigs were clipped in 2 areas (3x3 cm) on their backs. A dose of 0.1 grams of isoeugenol (100%) was applied to one of the areas, while the second area remained untreated. The irritation potential was read following contact with isoeugenol for 24 hours, and another application was administered every 24 hours (for a total of 3 applications, or 0.3 grams isoeugenol). After 72 hours, the animals were sacrificed and a thorough histopathological examination of the skin was performed to determine the relative irritancy. Isoeugenol was observed to be severely irritating to guinea pig skin [Motoyoshi et al., 1979].

**dermal, rabbits**

- The dorsal fur of 6 albino angora rabbits was clipped (3x3 cm areas). A dose of 0.1 grams of isoeugenol (100%) was applied to one of the exposed areas, and a second exposed area was treated with N-hexadecane (positive control). A third area was left untreated. The irritation potential was read following contact with isoeugenol for 24 hours, and another application was administered every 24 hours (for a total of 3 applications or 0.3 grams isoeugenol). After 72 hours, the animals were
sacrificed, and the total irritation potential was scored. Isoeugenol was found to be severely irritating to rabbit skin upon histopathological examination [Motoyoshi et al., 1979].

**dermal, miniature swine**

- Six Pittman-Moore Improved miniature swine were used to study isoeugenol-induced dermal irritation. The hair on the swines' backs was clipped, and a dose of 0.05 grams of isoeugenol (100%) was applied under a 15 mm diameter patch. The patches were secured for a 48-hour exposure period. After 48 hours the patches were removed, and the total irritation potential was scored. The results indicated that isoeugenol does not induce dermal irritation in swine [Motoyoshi et al., 1979].

C. Prechronic

1. Human Data

**dermal, human**

- Standard patch testing and photopatch testing were performed on a 40-year-old rockblaster of 10 years, who suddenly developed a severe itchy, confluent vesiculo-bullous reaction on his hands while working with dinitrotoluene. He was patch tested in duplicate using Finn Chambers technique with the ICDRG standard test series, including isoeugenol (2% in petrolatum), for 48 hours. One of the test areas was subsequently irradiated with 5 joules of ultraviolet light, and the results were read 48 hours after illumination. The individual exhibited a severe contact sensitivity reaction to isoeugenol. However, the case subject did not exhibit a photocontact allergy to this compound [Emtestam and Forbeck, 1985].

- Standard patch testing was performed on a 46-year-old female cookie handler who presented with a 2-week history of eczema of her hands from handling "Thin Mint" cookies which developed after the employee's duties were changed to include handling the "Thin Mint" line. After 48 hours, the woman reportedly had a positive reaction to an unspecified amount of isoeugenol, one of the compounds included in the standard patch test series. She did not exhibit a positive reaction to peppermint oil which was originally suspected to be the primary irritant. The authors indicated that she was able to handle other cookies without problems, and postulated that the concentration of the sensitizing components may be higher in the mint cookies than in other cookies [Spencer and Fowler, 1988].

- Standard patch testing was performed on a 47-year-old female pharmacist who had developed constant rhinitis and mild dermatitis around her eyes which had persisted for 18 months. She had daily contact with coal tar and bitter almond oil. Before the appearance of the dermatitis around her eyes she had used crayons on her eyelids. She tested strongly positive to isoeugenol, although she had no occupational exposure to pure isoeugenol [Rudzki and Grzywa, 1977].

Many other patch test studies have been performed to determine the incidence of contact allergy to isoeugenol. Table 9 summarizes the results of these patch tests.

- Table 10 represents the compilation of results from patch testing collected by members of the Soap and Detergent Association (SDA). The results of the survey indicate that isoeugenol has a very low potential for either eliciting pre-existing sensitization reactions or inducing hypersensitivity in subjects exposed to isoeugenol-containing consumer products. Five case subjects exhibited an induced reaction following multiple exposures. Data on the sensitization reactions in these 5 subjects are presented in Table 11 [Thompson et al., 1983].

2. Animal Data
The sensitization potency of isoeugenol was studied using 10 female Hartley albino guinea pigs. Fifty mg of a 10% solution of isoeugenol in petrolatum was applied (via a cloth on Torri's patch plaster) to a shaved portion of the nape of the animals' necks. The isoeugenol was removed after a 48-hour exposure. This procedure was repeated 3 times a week for 2 weeks. After the 2-week induction period, the guinea pigs' flanks were shaved, and isoeugenol was applied at concentrations of 0.1% and 1.0% for 48 hours. The degree of reaction was read 1, 24, 48 hours, and 1 week after the isoeugenol was removed. The results indicated that 40% (8/20) and 80% (16/20) of the animals exhibited sensitization to 0.1% and 1.0% isoeugenol, respectively, 72 hours after application [Itoh, 1982].

D. Chronic/Carcinogenicity

1. Human Data

No data were found in the literature on carcinogenicity or other chronic effects in humans.

2. Animal Data

No data were found in the literature on carcinogenicity or other chronic effects in animals.

E. Reproductive Effects and Teratogenicity

1. Human Data

No data were found in the literature on reproductive effects and teratogenicity in humans.

2. Animal Data

No data were found in the literature on reproductive effects and teratogenicity in animals.

F. Genetic Toxicology

1. Human Data

The following study concerning the genetic toxicology of isoeugenol in humans has been described. No other data were found in the literature.

in vitro, humans

Blood was collected from healthy, non-smoking volunteers for an in vitro study of the effects of cigarette smoke condensate upon sister-chromatid exchanges (SCE) in human lymphocytes. The weakly acidic semivolatile (WASV) fraction of cigarette smoke condensate, which was prepared using nonfiltered American blend cigarettes, was separated by preparative gel chromatography in to eleven subfractions. The chemical composition of these subfractions was determined, and the effects of the subfractions on SCE in cultured lymphocytes were investigated. Isoeugenol was found in subfraction F8, which contained as its main components phenol, 3-methylphenol, and 4-methylphenol.

The F8 fraction was found to increase significantly SCE induction in human lymphocytes (P<0.01). However, this effect was more significant in the other fractions tested. The effects of the individual components of Fraction 8 on SCE induction were also determined. The SCE induction ability of isoeugenol was found to be moderate at condensate concentrations of 0.25 mM (P<0.01) and 0.5 mM (P<0.001) [Jansson et al., 1986].
2. Prokaryotic Data

*Salmonella typhimurium*

- Isoeugenol was tested in a preincubation modification of the standard Ames test in *Salmonella typhimurium* TA1535, TA1537, TA98, TA100, and TA97 with and without Aroclor 1254-induced rat and hamster metabolic activation. This compound was found to be non-mutagenic at all concentrations tested (3.3, 10.0, 33.0, 100.0, 250.0, 333.0, and 800.0 _g/plate) [Mortelmans et al., 1986].

- In the standard Ames test, isoeugenol was tested for mutagenicity in *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537, and TA1538 in the presence and absence of metabolic activation. Isoeugenol was tested at doses of 60.0, 120.0, 300.0 and 600.0 _g/plate. Isoeugenol was observed to be non-mutagenic in all of the *Salmonella* strains tested [Sekizawa and Shibamoto, 1982].

- Mutagenicity tests were performed on *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98 in the presence and absence of metabolic activation. Isoeugenol was non-mutagenic in these strains of *Salmonella* at a concentration of 0.8 mg per plate [Nestmann, et al, 1980].

- In the Ames test, the mutagenic effects of isoeugenol were tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in the presence and absence of S9 liver metabolic activation. Isoeugenol was observed to be non-mutagenic at a concentration of 3.0 mmol/plate in all of the *Salmonella* strains tested [Florin et al., 1980].

- In the Ames test, the mutagenic effects of isoeugenol were tested in *Salmonella typhimurium* strains TA100, TA98, TA1535, TA1537, and TA1538 with metabolic activation. The tests were conducted at dose levels of 2.0, 20.0, and 200.0 mg/plate isoeugenol. Isoeugenol was observed to be non-mutagenic at all of the doses tested [Hsia et al., 1979].

- The urinary metabolites of isoeugenol were tested for mutagenicity in the Ames test using *Salmonella typhimurium* TA98 and TA100 in the presence of metabolic activation. To obtain the isoeugenol urinary metabolites, 2 Sprague-Dawley rats were administered a 0.5 ml dose of isoeugenol via a gastric tube. The urine was then collected for the next 24 hours and sterilized. Two ml of urine was set aside for a direct urine assay. The remainder of the urine sample was diluted with a phosphate buffer at pH 7.0, and incubated with a beta-glucuronidase preparation for 4 hours. The incubation sample was then extracted with ether. The ether extract, aqueous fraction, direct urine, and control urine samples were used for the assays. No mutagenic activity was observed in *Salmonella* from the direct urine or from the aqueous fractions of the ether extractions [Rockwell and Raw, 1979].

*Escherichia coli*

- Mutagenicity tests were performed on *Escherichia coli* WP2- uvrA, trp^- with and without S9 liver metabolic activation. Isoeugenol was tested at doses of 60.0, 120.0, 300.0, and 600.0 mg/plate. No mutagenic effects were observed [Sekizawa and Shibamoto, 1982].

- The SOS-inducing potency of 5.0 mM isoeugenol was tested in *Escherichia coli* strain PQ37 following nitrosation in a reaction mixture containing 10% ethanol. Isoeugenol was not found to be genotoxic in this test system in the absence of metabolic activation [Ohshima et al., 1989].

*Bacillus subtilis*

- In the DNA-repair test (rec assay) the mutagenic effects of isoeugenol were tested in *Bacillus subtilis* strains H17 Rec+ and M45 Rec-, without metabolic activation. Isoeugenol was tested at a dose of 0.8
mg/disk and was found to be genotoxic in this assay [Sekizawa and Shibamoto, 1982].

- In the DNA-repair test, isoeugenol was observed to be non-mutagenic in Bacillus subtilis strains H17 Rec+ and M45 Rec- at a dose of 22.0 mg/disk [Oda et al., 1978].

3. Eukaryotic Data

**Chinese hamster ovary cells**

- The effect of isoeugenol on SCE induction was tested in vitro at concentrations of 0, 10.0, 33.3, 100.0, and 333.0 mM in cultured Chinese hamster ovary cells (CHO K-1) with and without mitomycin C. Isoeugenol was found to be toxic at 333.0 mM. Isoeugenol did not have any influence on the cell cycle, nor did it have any effect upon SCE induction at any concentration tested [Sasaki et al., 1989].

**Saccharomyces cerevisiae**

- Isoeugenol was tested at an unspecified concentration for mutagenic effects in Saccharomyces cerevisiae strains D7 and XV185-14C without S9 metabolic activation. Isoeugenol did not induce tryptophan gene convertants in strain D7 or reversion of the histidine, homoserine, or tryptophan markers in strain XV185-14C, and was determined to be non-mutagenic in this test system [Nestmann and Lee, 1983].

G. Other Toxicological Effects

1. Immunotoxicity

a. Human Data

No data were found in the literature on immunotoxicity in humans.

b. Animal Data

**dermal, mice**

- Isoeugenol was applied to the backs of CBA/Ca mouse ears at concentrations of 0, 5, 10, and 25% for 3 consecutive days. The auricular (draining) lymph nodes were removed and weighed. The number of pyroninophilic cells were counted and lymphocyte proliferation was also determined, with and without interleukin-2. Isoeugenol (5, 10 and 25%) was found to elicit an immunological response in the draining lymph nodes which was characterized by lymphocyte proliferation [Kimber and Weisenberger, 1989].

2. Neurotoxicity

No data were found in the literature on neurotoxicity in humans or animals.

3. Biochemical Toxicology

a. Human Data

No data were found in the literature on the biochemical toxicology of isoeugenol in humans.

b. Animal Data
The UDP-Glucuronosyltransferase (UDPGT) activities of hydroxy-coumarins, monoterpenoid alcohols, and alkylphenols, including isoeugenol, were measured in liver microsomes from Wistar rats induced by either phenobarbital or 3-methylcholanthrene, or in non-induced controls which received sodium chloride or oil only. The UDPGT activity of 0.25 mM isoeugenol was 1.5 times the control value, in the presence of phenobarbital, and 2.39 times the control value in the presence of 3-methylcholanthrene.

The UDPGT activity of isoeugenol and the effect of induction by phenobarbital were also studied using Gunn rats, which have partially reduced UDPGT activity and completely lack UDPGT-bilirubin activity. The UDPGT activity of 0.25 mM isoeugenol was induced in the presence of phenobarbital to 3.84 times control value.

The UDPGT activity of isoeugenol was also studied in the presence and absence of phenobarbital using guinea pigs of unspecified strain. The UDPGT activity of 0.175 mM isoeugenol was 1.6 times control value in the presence of phenobarbital [Boutin et al., 1985].

4. Cytotoxicity

**in vitro, humans**

- Cultured human diploid embryonic lung fibroblasts (line MRC-5) were used to determine the damaging effects of tobacco smoke components, including isoeugenol. A dose of 25.0 mM isoeugenol was added to the cells for a 30-minute incubation period. The severity of damage to the plasma membrane was determined by the percentage of nucleotides released from the plasma membrane: high (>70%), moderate (70-48%), and no damage (<15%) nucleotide release. The study indicated that the plasma membrane was severely damaged by isoeugenol based on a 90% nucleotide release [Thelestam et al., 1980].

**in vitro, humans, hamsters, chickens**

- The relative cytotoxicity of isoeugenol was determined by compiling the data from cytotoxicity studies involving the following 4 parameters: inhibition of cell growth, inhibition of oxidative metabolism, plasma membrane damage, and ciliotoxicity.

Isoeugenol's ability to inhibit cell growth (CG) was determined using ascites sarcoma BP 8 cells. A dose of 1.0 mM of isoeugenol was added to the BP 8 cells and incubated for 48 hours. The rate of growth was then compared to a control to determine isoeugenol's ability to inhibit growth and expressed as a percentage. Isoeugenol was found to be a strong cell growth inhibitor (See ACG, Table 12).

Brown fat cells from hamsters were used to determine the ability of isoeugenol to inhibit oxidative metabolism (OM). The brown fat cells were treated with 0.6 _M norepinephrine to increase the rate of oxygen consumption. A dose of 1.0 mM of isoeugenol was added to the fat cells and incubated for 5 minutes. The toxicity was determined by comparing the norepinephrine-induced oxygen consumption, after addition of isoeugenol, to that of the control and expressing the ratio as a percentage. Isoeugenol was found to strongly inhibit oxidative metabolism (See AOM, Table 12).

The ability of isoeugenol to induce plasma membrane damage (MD) was determined by the percentage of cytoplasmic nucleotide leakage. Isoeugenol was added as a 25.0 mM solution in either ethanol or
dimethyl sulfoxide (author did not specify which of these solvents was used) to cultured human diploid embryonic lung fibroblasts (line MRC-5) and incubated for a period of 30 minutes. Isoeugenol was found to have a strong damaging effect on plasma membranes (See A\textsuperscript{MD}, Table 12).

The ability of isoeugenol to cause ciliostasis in embryo chicken trachea was used to measure ciliary activity (CA). A dose of 5.0 mM isoeugenol was added to the cultured trachea, and the time required for ciliostasis was recorded. The ciliotoxic effect was expressed as a percentage using time in minutes to ciliostasis when occurring within 60 minutes. Isoeugenol was determined to be a strong ciliary inhibitor (see A\textsuperscript{CA}, Table 12).

The relative toxicity (AT) of isoeugenol was determined to be high (see Table 12). This value, as well as the individual cytotoxicity values are expressed on a 10-point scale (0: 0-9%, 1: 10-19%, 2: 20-29%, 3: 30-39%, 4: 40-49%, 5: 50-59%, 6: 60-69%, 7: 70-79%, 8: 80-89%, 9: 90-100%) [Curvall, et al., 1984].

\textit{in vitro, chickens}

- The length of time required for ciliostasis in cultured embryo chicken trachea was measured to determine the ciliotoxicity of isoeugenol. A single dose of 5.0 mM isoeugenol was added to the culture and the time required for ciliostasis was reported. The study indicated that isoeugenol is a strong inhibitor of ciliary movement because ciliostasis occurred 6 minutes after dosing [Pettersson \textit{et al.}, 1982].
VI. STRUCTURE ACTIVITY RELATIONSHIPS

Isoeugenol is structurally related to eugenol, safrole, isosafrole, estragole [NCI, 1984b], and methyleugenol (see below) [NTP, 1991].

[Chemical structures of Isoeugenol, Estragole, Eugenol, Safrole, Isosafrole, and Methyleugenol]

IARC (1976) reported that safrole and isosafrole are carcinogenic in mice and rats; these chemicals produced liver tumors following oral administration. Subcutaneous injection of safrole produced liver and lung tumors in male infant mice [IARC, 1976].

Estragole has been found to induce hepatomas in male and female CD-1 mice following administration by gavage twice weekly for 5 weeks (total dose 25 mmol/g). In this study, mice were autopsied at 11-14 months. This compound also induced hepatomas following oral administration (0.23%; 0.46%) for 12 months in female CD-1 mice. In male CD-1 mice, estragole caused hepatocellular carcinomas following subcutaneous administration on days 1, 8, 15, and 22 after birth (total dose 4.43 and 5.19 mmol) and after subcutaneous administration (total dose 4.43 and 5.19 mmol) in male CD-1 mice in a 15 month study. Intraperitoneal injection of this compound induced liver hepatomas in male C3H and B6C3F1 mice in a 12
month (total does 9.45 mmol) and 13-18 month (total dose 4.75 mmol) study, respectively [CCRIS, 1991].

NTP conducted feeding carcinogenicity studies of eugenol in male and female F344/N rats and male and female B6C3F1 mice. For male mice, there was equivocal evidence of carcinogenicity based on increased incidences (P<0.05) of both carcinomas and adenomas in the liver of animals in the low dose group (3,000 ppm) compared to the control group. A significant increase in hepatic neoplasms was not observed in the high dose animals. For female mice, there was equivocal evidence of carcinogenicity based on an increase in the combined incidences of hepatocellular carcinomas or adenomas in the high dose group (6,000 ppm) compared to the control group [NTP, 1983].

In a 13-week cumulative toxicity study on eugenol, using male and female F344/N rats and B6C3F1 mice, no eugenol-related gross or histopathologic effects were observed in either species [NTP, 1983].

NTP has conducted a subchronic toxicity study (gavage) of methyleugenol in male and female Fischer 344 rats and male and female B6C3F1 mice. For the mice, possible treatment-related gross observations involved the liver, stomach and spleen and thin carcass of some animals in the high dose groups (1,000 mg/kg). Increased liver weights were observed in all test groups in both sexes of mice and were considered to be treatment-related. Primary pathologic effects included atrophy, degeneration, necrosis, edema, and mitotic alteration in the glandular stomach and cytological alteration, necrosis, biliary tract hyperplasia, and subacute inflammation in the liver. Nasal mucosal degeneration was also observed in mice from the high dose groups.

For the rats, possible treatment-related gross observations involved the liver (all test groups) and testes (1,000 mg/kg only) of male rats and the liver (300 mg/kg and 1,000 mg/kg only) and uterus (1,000 mg/kg only) of female rats. Primary pathologic effects included inflammation, degeneration, atrophy, and hyperplasia of the glandular stomach and cytologic alteration, biliary tract hyperplasia, cytomegaly, and multiple mixed cell focus of the liver. Histopathologic alterations of the kidney, testes or uterus, adrenal gland, salivary gland, and spleen (male rats only) were also observed [NTP, 1989; NTP, 1990].
VII. REFERENCES


BRS Information Technology, HZDB Database, 1990.


National Cancer Institute (NCI), 1984a, Letter from Dr. T. Cameron, Chairman, Chemical Selection Working Group, National Cancer Institute, to Dr. D. Canter, NTP.


National Toxicology Program (NTP), Technical Report Series No. 233, Carcinogenesis Studies of Eugenol (CAS No. 97-53-0) in F344/N Rats and B6C3F1 Mice (Feed Studies), U.S. Department of Health and Human Services, 1983.

National Toxicology Program (NTP), Abstracts of Subchronic Gavage Studies in Fischer-344 Rats and B6C3F1 Mice. February, 1989.


National Toxicology Program (NTP), Personal Communication with Dr. William Eastin, February 15, 1991.


## APPENDIX I. ON-LINE DATABASES SEARCHED

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<td>October, 1990</td>
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APPENDIX II. SAFETY INFORMATION

HANDLING AND STORAGE

Isoeugenol is stable under normal laboratory conditions.

EMERGENCY FIRST AID PROCEDURES

**Eye:** First check the victim for contact lenses and remove if present. Flush victim's eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control center. Do not put any ointments, oils, or medication in the victim's eyes without specific instructions from a physician. Immediately transport the victim to a hospital even if no symptoms (such as redness or irritation) develop.

**Skin:** IMMEDIATELY flood affected skin with water while removing and isolating all contaminated clothing. Gently wash affected skin areas thoroughly with soap and water. If symptoms such as inflammation or irritation develop, IMMEDIATELY call a physician or go to a hospital for treatment.

**Inhalation:** IMMEDIATELY leave the contaminated area and take deep breaths of fresh air. If symptoms (such as wheezing, coughing, shortness of breath, or burning in the mouth, throat, or chest) develop, call a physician and be prepared to transport the victim to a hospital.

Provide proper respiratory protection to rescuers entering an unknown atmosphere. Whenever possible, Self-Contained Breathing Apparatus (SCBA) should be used.

**Ingestion:** If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and IMMEDIATELY call a hospital or poison control center. Be prepared to transport the victim to a hospital if advised by a physician.

If the victim is convulsing or unconscious, do not give anything by mouth, ensure that the victim's airway is open, and lay the victim on his/her side with the head lower than the body. DO NOT INDUCE VOMITING. IMMEDIATELY TRANSPORT THE VICTIM TO A HOSPITAL.

PROTECTIVE EQUIPMENT

**Eye:** Splash-proof safety goggles

**Gloves:** Two pairs of dissimilar protective gloves shall be worn when handling the neat chemical, otherwise one pair. When contact with this chemical has been known to occur, change gloves immediately.

**Clothing:** Minimally, a disposable laboratory suit (e.g. Tyvek ®) shall be worn, as specified in the most current NTP Statement of Work or NTP Health and Safety Minimum Requirements.

**Respiratory** A NIOSH-approved chemical cartridge respirator with an Protection: organic vapor cartridge.

EXTINGUISHANT

Dry chemical, carbon dioxide or halon extinguisher

MONITORING PROCEDURES
There is no NIOSH analytical method reported in the NIOSH Manual of Analytical Methods for isoeugenol.

**SPILLS AND LEAKAGE**

Persons not wearing the appropriate protective equipment and clothing shall be restricted from areas of spills until cleanup has been completed. When exposure to unknown concentrations may occur, air-purifying respirators may not be used. Chemical cartridge respirators with organic vapor cartridges may not be used when airborne concentrations exceed 1000 ppm.

If isoeugenol is spilled the following steps shall be taken:

1. In order to prevent dust formation, use moistened paper towels to clean up a solid spill. Avoid dry sweeping.
2. If a liquid solution is spilled, use vermiculite, sodium bicarbonate, sand, or paper towels to contain and absorb the spill.
3. Clean the spill area with dilute alcohol (approximately 60-70%) followed by a strong soap and warm water washing.
4. Dispose of all absorbed material as hazardous waste.

**DECONTAMINATION OF LABORATORY EQUIPMENT**

**TDMS Terminal:** Whenever feasible, a protective covering (e.g., plastic wrap) shall be placed over the keyboard when in use.

**General Equipment:** Before removing general laboratory equipment (i.e., lab carts, portable hoods and balances) from animal dosing rooms and/or chemical preparation areas, a decontamination process shall be conducted in addition to routine housekeeping procedures.

**WASTE MANAGEMENT AND DISPOSAL PROCEDURES**

**Waste Management:** If an inhalation study is to be conducted, all exhaust air from the inhalation chamber must be cleaned with appropriate air cleaning devices unless the laboratory has informed local and state air pollution regulatory agencies of both the laboratory’s operating practices and the potential hazards of the chemicals in use. Compliance with all federal, state, and local air pollution laws and regulations is required. A specific air cleaning system design must consider the specific conditions of the laboratory (e.g., air flow rates and volumes, mixing of exhaust streams, size of inhalation chamber) and the dosing regimen selected. Air cleaning systems designs must be described by the laboratory and approved by the NTP Office of Laboratory Health and Safety.

**Waste Disposal:** Securely package and label, in double bags, all waste material. All potentially contaminated material (i.e., carcasses, bedding, disposable cages, labware) shall be disposed of by incineration in a manner consistent with federal (EPA), state, and local regulations or disposed of in a licensed hazardous waste landfill.
Table 1. Net Quantity of Isoeugenol and Eugenol Exported to the United States by Country 1985-1988

<table>
<thead>
<tr>
<th>Source</th>
<th>Countries</th>
<th>Net Quantity (lbs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S. Imports for Consumption * 1985</td>
<td>France</td>
<td>40,958</td>
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<td></td>
<td>Singapore</td>
<td>87,303</td>
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<td></td>
<td>Indonesia</td>
<td>163,141</td>
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<tr>
<td></td>
<td>Other</td>
<td>6,370</td>
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<tr>
<td></td>
<td>Total</td>
<td>297,772</td>
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<tr>
<td>U.S. Imports for Consumption 1986</td>
<td>France</td>
<td>26,168</td>
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<td></td>
<td>Spain</td>
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<td></td>
<td>Indonesia</td>
<td>202,933</td>
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<td></td>
<td>Japan</td>
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<tr>
<td></td>
<td>Other</td>
<td>11,454</td>
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<tr>
<td></td>
<td>Total</td>
<td>276,492</td>
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<tr>
<td>U.S. Imports for Consumption 1987</td>
<td>Singapore</td>
<td>216,050</td>
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<td>Indonesia</td>
<td>308,205</td>
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<td>Japan</td>
<td>25,132</td>
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<td></td>
<td>Other</td>
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<td></td>
<td>Total</td>
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<td></td>
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<tr>
<td></td>
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<td>1,053,156</td>
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* Imports for consumption is a measure of the total volume of merchandise that has cleared through Customs, whether such merchandise enters consumption channels immediately, or is withdrawn for consumption from warehouses under Customs custody, or is entered into U.S. Customs territory from Foreign Trade Zones.
Table 2. Concentrations of Isoeugenol in Foods and Beverages.

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<thead>
<tr>
<th>Product</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Non-alcoholic beverages</td>
<td>3.7 ppm</td>
</tr>
<tr>
<td>Ice cream, ices, etc.</td>
<td>3.8 ppm</td>
</tr>
<tr>
<td>Candy</td>
<td>5 ppm</td>
</tr>
<tr>
<td>Baked goods</td>
<td>11 ppm</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>0.3 - 1,000 ppm</td>
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<tr>
<td>Condiments</td>
<td>1.0 ppm</td>
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Table 3. Lotion Composition

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</tr>
<tr>
<td>ethyl alcohol</td>
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<td>methyl p-hydroxybenzoate</td>
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<td>polyoxyethylene olein-alcohol ether</td>
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<td>distilled water</td>
<td>70.0%</td>
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Reference: Jimbo et al., 1983
<table>
<thead>
<tr>
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<tr>
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</tr>
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</tr>
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Reference: Jimbo *et al.*, 1983
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</thead>
<tbody>
<tr>
<td>squalane</td>
<td>20.0%</td>
</tr>
<tr>
<td>petrolatum</td>
<td>5.0%</td>
</tr>
<tr>
<td>cetyl alcohol</td>
<td>5.0%</td>
</tr>
<tr>
<td>glycercy monostearate</td>
<td>3.0%</td>
</tr>
<tr>
<td>polyoxyethylene sorbitan monooleate</td>
<td>2.0%</td>
</tr>
<tr>
<td>ethyl p-hydroxybenzoate</td>
<td>0.2%</td>
</tr>
<tr>
<td>butyl p-hydroxybenzoate</td>
<td>0.2%</td>
</tr>
<tr>
<td>glycerin</td>
<td>5.0%</td>
</tr>
<tr>
<td>distilled water</td>
<td>59.6%</td>
</tr>
</tbody>
</table>

Reference: Jimbo et al., 1983
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>squalane</td>
<td>30.0%</td>
</tr>
<tr>
<td>petrolatum</td>
<td>15.0%</td>
</tr>
<tr>
<td>wax</td>
<td>5.0%</td>
</tr>
<tr>
<td>bees wax</td>
<td>10.0%</td>
</tr>
<tr>
<td>sorbitan sesquioleate</td>
<td>4.5%</td>
</tr>
<tr>
<td>polyoxyethylene sorbitan monooleate</td>
<td>0.5%</td>
</tr>
<tr>
<td>ethyl p-hydroxybenzoate</td>
<td>0.1%</td>
</tr>
<tr>
<td>butyl p-hydroxybenzoate</td>
<td>0.1%</td>
</tr>
<tr>
<td>glycerin</td>
<td>5.0%</td>
</tr>
<tr>
<td>distilled water</td>
<td>24.8%</td>
</tr>
</tbody>
</table>

Reference: Jimbo et al., 1983
### Table 7. O/W Type Foundation Composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>propylene glycol</td>
<td>5.0%</td>
</tr>
<tr>
<td>polyethylene glycol 4000</td>
<td>5.0%</td>
</tr>
<tr>
<td>squalane</td>
<td>10.0%</td>
</tr>
<tr>
<td>cetyl alcohol</td>
<td>0.8%</td>
</tr>
<tr>
<td>stearic acid</td>
<td>2.5%</td>
</tr>
<tr>
<td>polyoxyethylene oleate</td>
<td>1.0%</td>
</tr>
<tr>
<td>sorbitan sesquioleate</td>
<td>1.0%</td>
</tr>
<tr>
<td>ethyl p-hydroxybenzoate</td>
<td>0.1%</td>
</tr>
<tr>
<td>butyl p-hydroxybenzoate</td>
<td>0.1%</td>
</tr>
<tr>
<td>titanium dioxide</td>
<td>9.5%</td>
</tr>
<tr>
<td>talc</td>
<td>5.72%</td>
</tr>
<tr>
<td>kaolin</td>
<td>3.8%</td>
</tr>
<tr>
<td>red iron oxide</td>
<td>0.26%</td>
</tr>
<tr>
<td>yellow iron oxide</td>
<td>0.62%</td>
</tr>
<tr>
<td>black iron oxide</td>
<td>0.1%</td>
</tr>
<tr>
<td>Mg-Al-silicate</td>
<td>1.0%</td>
</tr>
<tr>
<td>triethanolamine</td>
<td>1.25%</td>
</tr>
<tr>
<td>sodium hexametaric acid</td>
<td>0.03%</td>
</tr>
<tr>
<td>distilled water</td>
<td>52.25%</td>
</tr>
</tbody>
</table>

Reference: Jimbo et al., 1983
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>mica</td>
<td>21.4%</td>
</tr>
<tr>
<td>kaolin</td>
<td>10.0%</td>
</tr>
<tr>
<td>titanium oxide</td>
<td>15.0%</td>
</tr>
<tr>
<td>red iron oxide</td>
<td>0.5%</td>
</tr>
<tr>
<td>yellow iron oxide</td>
<td>1.0%</td>
</tr>
<tr>
<td>black iron oxide</td>
<td>0.1%</td>
</tr>
<tr>
<td>squalane</td>
<td>42.8%</td>
</tr>
<tr>
<td>sorbitan sesquioleate</td>
<td>0.9%</td>
</tr>
<tr>
<td>carnauba wax</td>
<td>1.0%</td>
</tr>
<tr>
<td>hard paraffin</td>
<td>7.0%</td>
</tr>
</tbody>
</table>

Reference: Jimbo *et al.*, 1983
<table>
<thead>
<tr>
<th>Case Subjects/Number</th>
<th>Isoeugenol Preparation</th>
<th>Conc. of Isoeugenol in Patch Test</th>
<th>Number of Positive/Patch-Test Subjects (time)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/64</td>
<td>FMC</td>
<td>1% PET</td>
<td>15/64 (NS)</td>
<td>Enders et al., 1989</td>
</tr>
<tr>
<td>Women/98</td>
<td>FMC</td>
<td>1% PET</td>
<td>12/98 (NS)</td>
<td>Enders et al., 1989</td>
</tr>
<tr>
<td>Patients with known positive reaction to Fragrance Mixture/32</td>
<td>FMC</td>
<td>8% PET</td>
<td>8/32 (48 &amp; 72 hrs.)</td>
<td>Roesyanto-Mahadi et al., 1990</td>
</tr>
<tr>
<td>Patients with Cosmetic Dermatitis and Melanosis/155</td>
<td>FMC</td>
<td>5% PET</td>
<td>8/155 (1,24,168,336 hrs.)</td>
<td>Itoh, 1982</td>
</tr>
<tr>
<td>Patients with Non-Cosmetic contact Dermatitis and Eczema/159</td>
<td>FMC</td>
<td>5% PET</td>
<td>11/159 (1,24,168,336 hrs.)</td>
<td>Itoh, 1982</td>
</tr>
<tr>
<td>Patient with Non-Inflammatory Skin Disorders/48</td>
<td>FMC</td>
<td>5% PET</td>
<td>1/48 (1,24,168,336 hrs.)</td>
<td>Itoh, 1982</td>
</tr>
<tr>
<td>Patients with Positive Reaction to Fragrance Mixture/42</td>
<td>FMC</td>
<td>NS</td>
<td>19/42 (NS) (NS)</td>
<td>Rudzki and Grzywa, 1986</td>
</tr>
<tr>
<td>Dermatology patients/2,461</td>
<td>FMC</td>
<td>2% PET</td>
<td>48/2,461 (NS) (NS)</td>
<td>Larsen, 1985</td>
</tr>
<tr>
<td>Patients with Eczematous Dermatitis/3,037</td>
<td>FMC</td>
<td>1% PET</td>
<td>6/3,037 (48 &amp; 72 hrs.)</td>
<td>Angelini et al., 1985</td>
</tr>
<tr>
<td>Allergy Patients/179</td>
<td>FMC</td>
<td>8% PET</td>
<td>36/178 (48 &amp; 72 hrs.)</td>
<td>DeGrout et al., 1985</td>
</tr>
<tr>
<td>Allergy Patients/122</td>
<td>FMC</td>
<td>NS</td>
<td>4/122 (NS)</td>
<td>Asoh and Sugai, 1985</td>
</tr>
<tr>
<td>Allergy Patients/242</td>
<td>FMC</td>
<td>2.8% PET</td>
<td>36/242 (NS)</td>
<td>Van Joost et al., 1985</td>
</tr>
<tr>
<td>Dermatology patients/5,202</td>
<td>FMC</td>
<td>NS</td>
<td>36/242 (NS)</td>
<td>Van Joost et al., 1985</td>
</tr>
<tr>
<td>Patients with Positive Reaction to Oak Moss/31</td>
<td>FMC</td>
<td>NS</td>
<td>9/31 (48 &amp; 96 hrs.)</td>
<td>Goncaloet al., 1988</td>
</tr>
<tr>
<td>Patients with Eczematous Dermatitis/2,461</td>
<td>FMC</td>
<td>2% PET</td>
<td>48/2461 (NS)</td>
<td>Calnan et al., 1980</td>
</tr>
<tr>
<td>* Patients with strong reaction to isoeugenol/8</td>
<td></td>
<td>5% PET</td>
<td>8/8 (72 hrs.)</td>
<td>Itoh, 1980</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1% PET</td>
<td>5/8 (72 hrs.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2% PET</td>
<td>4/8 (72 hrs.)</td>
<td></td>
</tr>
</tbody>
</table>

*This study was part of a study in which patients who exhibited reactions to isoeugenol were tested for cross-reactivity to other phenolic compounds.

FMC = Fragrance Mixture Component  
PET = Petrolatum  
NS = Not Specified  
NA = Not Applicable
Table 10. Human Sensitization Survey

<table>
<thead>
<tr>
<th>Case Number</th>
<th>No. of Subjects</th>
<th>Isoeugenol Preparation</th>
<th>Conc. of isoeugenol in Patch Test (%)</th>
<th>Test Method</th>
<th>Elicited</th>
<th>Induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>504</td>
<td>Personal Care</td>
<td>2 x 10^-2 - 5 x 10^-2</td>
<td>HRIPT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>Personal Care</td>
<td>2 x 10^-2</td>
<td>HRIPT</td>
<td>0</td>
<td>1^a</td>
</tr>
<tr>
<td>3</td>
<td>2307</td>
<td>Personal Care</td>
<td>9 x 10^-6 - 6 x 10^-3</td>
<td>HRIPT or PPT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>Household</td>
<td>2 x 10^-2</td>
<td>HRIPT</td>
<td>0</td>
<td>1^b</td>
</tr>
<tr>
<td>5</td>
<td>612</td>
<td>Household</td>
<td>3 x 10^-7 - 1 x 10^-4</td>
<td>HRIPT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>56</td>
<td>Fragrance Blend</td>
<td>0.8</td>
<td>HRIPT</td>
<td>0</td>
<td>1^a</td>
</tr>
<tr>
<td>7</td>
<td>360</td>
<td>Fragrance Blend</td>
<td>5 x 10^-2 - 1 x 10^-1</td>
<td>HRIPT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>Fragrance Blend</td>
<td>5 x 10^-2</td>
<td>OCPT</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>Fragrance Blend</td>
<td>4 x 10^-2</td>
<td>HRIPT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>83</td>
<td>Fragrance Blend</td>
<td>4 x 10^-2</td>
<td>HRIPT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>840</td>
<td>Fragrance Blend</td>
<td>1 x 10^-2 - 3 x 10^-2</td>
<td>HRIPT</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>51</td>
<td>Fragrance Blend</td>
<td>1 x 10^-2</td>
<td>OCPT</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>1399</td>
<td>Fragrance Blend</td>
<td>6 x 10^-5 - 8 x 10^-3</td>
<td>HRIPT or PPT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>40</td>
<td>Neat</td>
<td>1.25%</td>
<td>HRIPT</td>
<td>0</td>
<td>1^b</td>
</tr>
<tr>
<td>15</td>
<td>41</td>
<td>Neat</td>
<td>1.25%</td>
<td>HRIPT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>38</td>
<td>Neat</td>
<td>1.8%</td>
<td>HRIPT</td>
<td>0</td>
<td>1^b</td>
</tr>
<tr>
<td>17</td>
<td>56</td>
<td>Neat</td>
<td>0.5%</td>
<td>HRIPT</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a - Reaction attributed to isoeugenol-eugenol mixture
b - Reaction attributed to isoeugenol
Neat - Isoeugenol not containing any other essential oils
HRIPT - Human Repeat Insult Patch Test
OCPT - 8-hour Occluded Patch Test
PPT - Prophetic Patch Test

Reference: Thompson et al., 1983
Table 11. Sensitization Reactions Attributable to Isoeugenol or Isoeugenol/Eugenol Mixtures

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Reaction Grades: Induction Phase</th>
<th>Reaction Grades: Challenge Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (§)</td>
<td>0 0 1 0 4 5/4 X/5 X/4 X/4</td>
<td>5/5 6/5</td>
</tr>
<tr>
<td>4 (//)</td>
<td>0 0 0 0 0 1 1 1 5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>6 (+)</td>
<td>0 0 1 3 X X 2/X 5/X X/X</td>
<td>1/3 2/3</td>
</tr>
<tr>
<td>14 (+)</td>
<td>0 0 0 0 0 0 0 0 3/4</td>
<td>6/0</td>
</tr>
<tr>
<td>16 (‡)</td>
<td>0 0 0 0 0 0 0 0 0 4/-</td>
<td>3/-</td>
</tr>
</tbody>
</table>

**Reaction Grades**

+ - Scoring system ranging from 0 (no evidence of irritation) to 6 (strong reaction extending well beyond area of contact)
// - Scoring system ranging from 0 (no visible reaction) to 5 (bullous reaction)
‡ - Scoring system ranging from 0 (no evidence of irritation) to 7 (strong reaction spreading beyond test site)
§ - Scoring system ranging from 0 (no evidence)
X - Patch test not applied

A double grade during the induction phase indicates that the patch was moved to a new, adjacent site. The first number is the grade for the new site; the second is the grade for the residual reaction at the old site.

A double grade during the challenge phase indicates that the site was read at two different times after removal of a challenge patch. Patch no. 1 was applied to the old site and patch no. 2 was applied to a new site.

Reference: Thompson et al., 1983
### Table 12. Toxicity Values for Isoeugenol in Four Test Systems

<table>
<thead>
<tr>
<th>Compound</th>
<th>A\textsuperscript{CG}</th>
<th>A\textsuperscript{OM}</th>
<th>A\textsuperscript{MD}</th>
<th>A\textsuperscript{CA}</th>
<th>A\textsuperscript{T}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoeugenol</td>
<td>9</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>8.5</td>
</tr>
</tbody>
</table>

ACG - Degree of inhibition of cell growth in ascites sarcoma BP 8 cells  
AOM - Inhibition of oxidative metabolism in hamster brown fat cells  
AMD - Membrane damage of human diploid embryonic lung fibroblasts  
ACA - Inhibition of ciliary activity using chicken embryo trachea (CA)  
AT - Mean activity

Reference: Curvall \textit{et al.}, 1984