

## SUMMARY OF DATA FOR CHEMICAL SELECTION

$\beta$ -Caryophyllene  
87-44-5

### BASIS OF NOMINATION TO THE CSWG

The nomination of  $\beta$ -caryophyllene to the CSWG is based on widespread human exposure and an unknown potential for adverse health effects from long-term exposure to this structurally unique compound.  $\beta$ -Caryophyllene came to the attention of the CSPG because of information supplied by the Food and Drug Administration (FDA) from a review of "GRAS" substances used as direct food additives. According to the FDA data,  $\beta$ -caryophyllene is found in 80 different food additives. The possible average daily intake from foods has been estimated to be >10 mg. In addition, nearly 45,000 workers are potentially exposed to  $\beta$ -caryophyllene in the workplace.

### SELECTION STATUS

ACTION BY CSWG: 12/10/97

#### Studies requested:

- Subchronic study
- Metabolism study
- Cell transformation
- *In vivo* micronucleus
- Mouse lymphoma assay (NCI Short-Term Test Program)

Priority: Moderately high

#### Rationale/Remarks:

- Coordinate testing with NCI's Division of Cancer Prevention
- Reconsider for carcinogenicity after subchronic study is completed

### INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

Dr. Dan Benz, Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration (FDA) and Dr. Ed Matthews (formerly with CFSAN) provided information on  $\beta$ -caryophyllene from the FDA Priority-Based Assessment of Food Additives (PAFA) database.

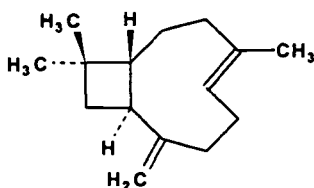
**FEB 24 1998**

Ms. Joellen Putnam, Scientific Project Manager, Flavor and Extract Manufacturers' Association (FEMA) provided a copy of the FEMA monograph on  $\beta$ -caryophyllene.

CHEMICAL IDENTIFICATION

<u>CAS Registry Number:</u>	87-44-5
<u>Chemical Abstract Service Name:</u>	Bicyclo(7.2.0)undec-4-ene, 4,11,11-trimethyl-8-methylene-, (1R-(1R*, 4E, 9S*))-(9CI)
<u>Synonyms and Trade Names:</u>	Caryophyllene; (-)- $\beta$ -caryophyllene; <i>trans</i> -caryophyllene
<u>Structural Class:</u>	Sesquiterpenoid

Structure, Molecular Formula and Molecular Weight:



$C_{15}H_{24}$

Mol. wt.: 204.36

Chemical and Physical Properties:

<u>Description:</u>	Liquid with a terpene odor midway between the odor of cloves and turpentine (Budavari, 1996)
<u>Boiling Point:</u>	129-130°C @ 14 mm Hg (Budavari, 1996)
<u>Flash Point:</u>	>200°F, CC (FEMA, 1997)
<u>Density:</u>	0.9075 g/cm <sup>3</sup> @ 20°C (Lide, 1995)
<u>Solubility:</u>	Insoluble in water; very soluble in benzene (Polarome International, 1997; Lide, 1995)
<u>Vapor Pressure:</u>	0.007 mm Hg @ 20°C (FEMA, 1997)

Technical Products and Impurities:  $\beta$ -Caryophyllene is available at purities of >80% and ~99% from TCI America and Fluka, respectively (TCI America, 1996; Fluka Chemical Corp., 1997).

## EXPOSURE INFORMATION

Production and Producers:  $\beta$ -Caryophyllene occurs in nature as a mixture with isocaryophyllene and  $\alpha$ -caryophyllene (Budavari, 1996).  $\beta$ -Caryophyllene can be prepared by isolation from clove leaf oil, clove stem oil, cinnamon leaf oil or pine oil fractions (FEMA, 1997). According to recent chemical catalogs and directories, caryophyllene is manufactured and/or distributed by Berje Inc., Fluka Chemical Corp., Penta Manufacturing Co., Polarome Manufacturing Co., Inc., Sigma Chemical Co., and TCI America (Van, 1995; TCI America, 1996; Fluka Chemical Corp., 1997; Sigma, 1997).

$\beta$ -Caryophyllene is listed as a chemical of commerce in the US International Trade Commission (USITC) publication *Synthetic Organic Chemicals, US Production and Sales* for the years 1983-1993 (USITC, 1984, 1985, 1986, 1987, 1988, 1989, 1990, 1991, 1993, 1994a, 1994b). The reporting companies were Biddle Sawyer Corp., Fragrance Resources, Inc., Fritch, Dodge & Olcott, Givaudan Corp., SMC Corp., Ungerer & Co., and Union Camp Corp.; but no production or sales quantities were included. According to the USITC, separate statistics were not published to avoid disclosure of individual company operations; however, the USITC reporting guidelines specify that each company's report of a chemical represents production of  $\geq 4,500$  kg [10,000 lbs] or sales  $\geq \$10,000$ .

$\beta$ -Caryophyllene is listed in the EPA's Toxic Substances Control Act (TSCA) Inventory (NLM, 1997).

Use Pattern:  $\beta$ -Caryophyllene has been used as a flavor and fragrance ingredient since the 1930s (Opdyke, 1973; FEMA, 1997). Its use in fragrances in the US in the early 1970s amounted to less than 20,000 lbs/year.  $\beta$ -Caryophyllene has been used in the following commercial products at the following concentrations (typical; maximum): soap, (0.01;

0.1%), detergent (0.001; 0.01%), creams and lotions (0.01; 0.1%), and perfume (0.04; 0.4%) (Opdyke, 1973).

β-Caryophyllene has the following reported food uses with associated use levels (typical; maximum ppm): baked goods (31.1; 42.5), frozen dairy (20.9; 26.6), meat products (10; 15), condiment relish (49.0; 70.8), soft candy (38.3; 52.3), gelatin pudding (69.3; 76.6), non-alcoholic beverages (23.5; 30.1), alcoholic beverages (2.6; 6.9), and chewing gum (293.3; 721.9). Levels of use of β-caryophyllene as a flavoring agent have been 1795 lbs in 1970, 1487 lbs in 1975, 4048 lbs in 1982 and 6358 lbs in 1987 (FEMA, 1997). In 1994, the annual consumption of β-caryophyllene as a flavoring agent was estimated at 10,633 lbs (FDA, 1994).

**Human Exposure:** There is potential for widespread, low-level exposures to β-caryophyllene in general and consumer populations resulting from its presence as a flavoring agent in foods and as a fragrance material. Caryophyllene is found in 80 direct food additive substances listed in the PAFA database (FDA, 1994). The possible average daily intake (PADI) of β-caryophyllene from foods has been estimated at 10.24 mg (FEMA, 1997). Food products containing β-caryophyllene are presented in Table 1.

Table 1. Food products containing β-Caryophyllene (in ppm)

Food	Lower Limit	Upper Limit	Food	Lower Limit	Upper Limit
Orange juice	0.01		Carrot	1.2	47.1
Lemon peel oil	2,000	3,000	Celery leaves (raw)	3.8	
Grapefruit juice	5.3	32.5	Celery root	0.08	0.4
Grapefruit peel oil	2,500	3,100	Anise	8,000	23,700
Mandarin peel oil	600		Cloves	10,300	16,000
Lime peel oil (coldpressed)	TRACE		Nutmeg	TRACE	
Lime peel oil (distilled)	TRACE		Pepper	2,300	10,000
Bilberry	0.002		Chicken (heated)	0.001	
Guava	0.0009	6.8	Green tea	2.8	
Raspberry	1.7		Mango	0.0003	2.3

From FEMA, 1997

The National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 44,746 workers, including 27,262 female workers, were potentially exposed to  $\beta$ -caryophyllene in the workplace. The NOES database does not contain information on the frequency, level or duration of exposure to workers of any chemicals listed therein (NLM, 1997).

Environmental Occurrence:  $\beta$ -Caryophyllene is found in nature as a constituent of the essential oils of clove, cinnamon leaves, and copaiba balsam and in minor quantities in various other essential oils, especially lavender (Opdyke, 1973).

$\beta$ -Caryophyllene has been identified as a volatile organic compound emitted by vegetation into the atmosphere (Guenther *et al.*, 1994). Sesquiterpenoids are introduced into the atmosphere by direct volatilization from plants and from biomass burning.  $\beta$ -Caryophyllene has also been identified in sediments of a coastal lagoon (Elias *et al.*, 1997).

$\beta$ -Caryophyllene has been reported in the emissions from household liquid floor wax and paste wax for leather as well as in the emissions of kitchen and garden waste (Knöppel & Schauenburg, 1989; Wilkins & Larsen, 1995).

$\beta$ -Caryophyllene is a major constituent in clove cigarette smoke, delivering up to 2 mg caryophyllene per cigarette (Wise & Guerin, 1986). It has also been identified in the volatile oil of fresh and dried marijuana buds at levels up to 5.45% of the oil (Ross & ElSohly, 1996).

Regulatory Status: No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of  $\beta$ -caryophyllene.  $\beta$ -caryophyllene was not on the American Conference of Governmental Industrial

Hygienists (ACGIH) list of compounds for which recommendations for a threshold limit value (TLV) or biological exposure index (BEI) are made.  $\beta$ -Caryophyllene has been granted “generally recognized as safe” (GRAS) status by FEMA and is approved by the FDA for food use (Opdyke, 1973; FEMA, 1997).

## EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposure to  $\beta$ -caryophyllene and cancer risk in humans were identified in the available literature.

At concentrations up to 4%,  $\beta$ -caryophyllene did not cause skin irritation or sensitization in human subjects (Opdyke, 1973; FEMA, 1997).

Animal Data: No 2-year carcinogenicity studies of  $\beta$ -caryophyllene in animals were identified in the available literature. Toxicity information was limited to acute data.

Both the acute oral LD<sub>50</sub> in rats and the acute dermal LD<sub>50</sub> in rabbits exceeded 5 g/kg (Opdyke, 1973). Intratracheal doses of 12, 24, or 48 mg/kg of  $\beta$ -caryophyllene were not toxic to the lungs of F344 male rats (LaVoie *et al.*, 1986).

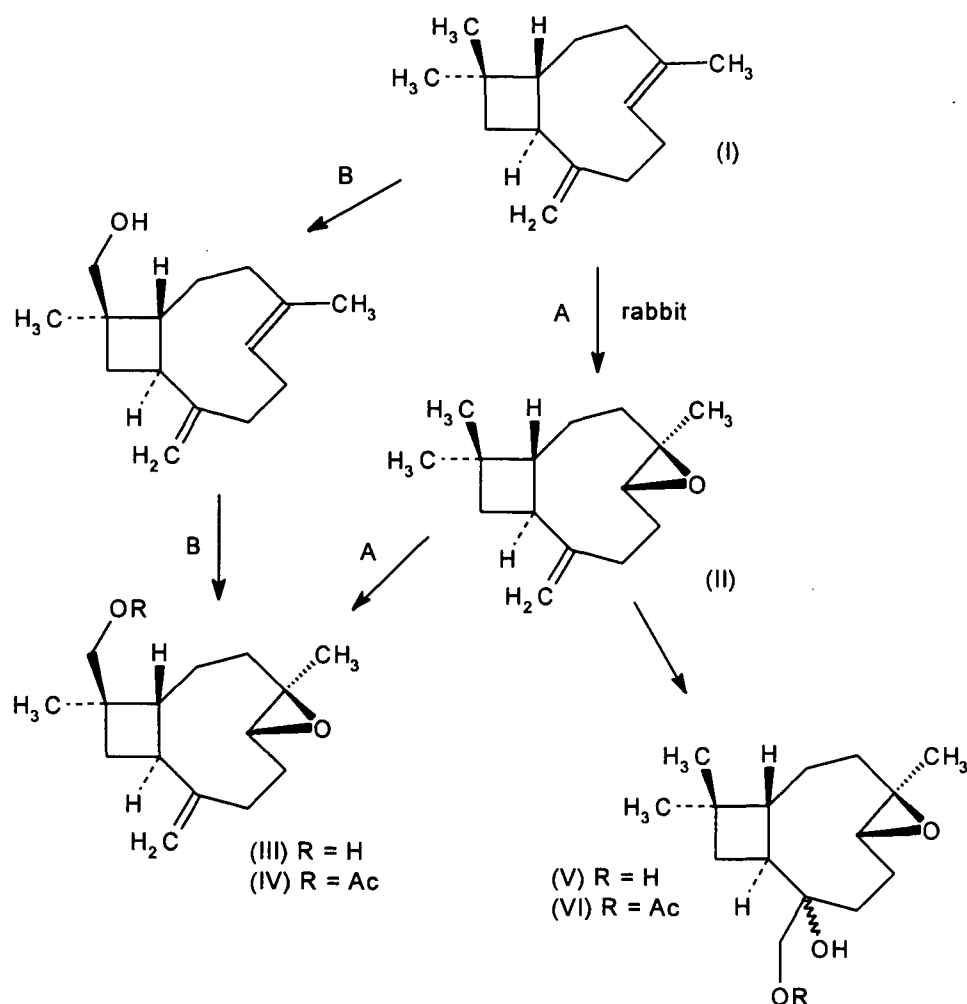
Short-Term Tests: At concentrations up to 150,000  $\mu$ g/plate,  $\beta$ -caryophyllene was not mutagenic in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 both with and without activation.  $\beta$ -Caryophyllene was also inactive in an unscheduled DNA synthesis assay using rat hepatocytes and doses up to 10,000  $\mu$ g/ml (Heck *et al.*, 1989; FEMA, 1997).

The frequency of spontaneous sister chromatid exchanges (SCE) in Chinese hamster ovary K-1 cells was not affected by  $\beta$ -caryophyllene concentrations up to 1,000  $\mu$ M. The same doses also failed to increase the frequency of mitomycin C-induced SCEs (Sasaki *et al.*, 1989).

Caryophyllene ( $\beta$  not specified) inhibited the mutagenic activity of aflatoxin B<sub>1</sub> in *Salmonella typhimurium* strains TA98 and TA100. A  $\beta$ -caryophyllene concentration of 3% produced a 72% inhibition of aflatoxin B<sub>1</sub> mutagenicity in TA100 (Kim *et al.*, 1992).



Metabolism: Asakawa and coworkers (1981,1986) characterized the metabolism of  $\beta$ -caryophyllene (I) in rabbits as shown in the following scheme.



The main metabolite, (10S)-(-)-14-hydroxycaryophyllene-5,6-oxide (III), was separated as an acetate, (10S)-(-)-14-acetoxycaryophyllene-5,6-oxide (IV), using column chromatography by n-hexane-ethyl acetate. A second acetate, identified as (-)-caryophyllene-5,6-oxide-2,12-diol monoacetate (VI), was the hydroxyacetate derivative of an additional metabolite, caryophyllene-5,6-oxide-2,12-glycol (V). The metabolic pathway shown as A was confirmed by administering (-)-caryophyllene-5,6-oxide (II) to rabbits. After being acetylated compound IV was identified and compound III was

established as the major metabolite. The authors note that the presence of the route B remains to be clarified and that route A may be favorable to route B since the oxide (II) is often found in essential oils. The stereoselective biohydroxylation of the *gem*-dimethyl group on the four-membered ring in mammals had not previously been reported.

Other Biological Effects: Several studies have documented the anti-inflammatory, cytoprotective, and enzyme-inducing activities of  $\beta$ -caryophyllene, as well as its *in vitro* cytotoxicity against several solid tumor cells.

In the rat hindpaw edema model induced by the phlogogen agents, carrageenan or prostaglandin E<sub>1</sub>,  $\beta$ -caryophyllene showed anti-inflammatory activity against both agents in female Wistar rats.  $\beta$ -Caryophyllene doses of 150 or 300 mg/kg and 300 or 600 mg/kg were orally administered 15 minutes prior to injection of carrageenan and prostaglandin E<sub>1</sub>, respectively.  $\beta$ -Caryophyllene also reduced carrageenan-induced inflammation in adrenalectomized animals. The authors note that arachidonic acid metabolism acts as a mediator of the inflammatory response induced by carrageenan. Therefore, a component of the anti-inflammatory activity of  $\beta$ -caryophyllene may be due to inhibition of prostaglandin synthesis and release, and also to its action, as shown by the activity against prostaglandin E<sub>1</sub>-induced edema (Martin *et al.*, 1993).

Tambe and coworkers (1996) demonstrated that  $\beta$ -caryophyllene, given orally but not intraperitoneally, was capable of protecting rats from the induction of gastric mucosal injuries by necrotizing agents such as ethanol and strong acid, without affecting gastric acid secretion.  $\beta$ -Caryophyllene administered at 1-150 mg/kg 15 minutes prior to ethanol administration protected the gastric mucosa of Sprague-Dawley rats in a dose-dependent manner. Complete suppression of ulcerogenesis was seen at doses of 25 mg/kg or more. The antiulcerogenic activity of  $\beta$ -caryophyllene was attributed to the potentiation of defensive factors via gastric cytoprotection.

The glutathione S-transferase (GST)-inducing activity of  $\beta$ -caryophyllene was determined in the liver, forestomach, and small intestine mucosa of female A/J mice. At the 20 mg dose,  $\beta$ -caryophyllene increased the GST activity 2.6 times more than the control in the liver and 3.0 times over the control in the small intestine mucosa. No significant increase of GST activity was observed in the forestomach. Induction of GST activity by anticarcinogenic compounds is believed to be a major mechanism for carcinogen detoxification (Zheng *et al.*, 1992).

$\beta$ -Caryophyllene exhibited significant *in vitro* cytotoxicity against human epithelioid cervix carcinoma (HeLa) cells and human breast carcinoma (BT-20) cells. The respective  $IC_{50}$ s were 3.86  $\mu$ g/ml and 3.92  $\mu$ g/ml. Moderate cytotoxicity was also noted against human melanoma skin cells (HTB-140) and mouse melanoma cells (B-16) (Kubo *et al.*, 1996).

Structure Activity Relationships: Caryophyllene is a unique molecule; closely related structural counterparts were not found.

## REFERENCES

- Asakawa, Y., Ishida, T., Toyota, M. & Takemoto, T. (1986) Terpenoid biotransformation in mammals IV. Biotransformation of (+)-longifolene, (-)-caryophyllene, (-)-caryophyllene oxide, (-)-cyclocolorone, (+)-nootkatone, (-)-elemol, (-)-abietic acid and (+)-dehydroabietic acid in rabbits. *Xenobiotica*, **16**(8), 753-767
- Asakawa, Y., Taira, Z., Takemoto, T., Ishida, T., Kido, M. & Ichikawa, Y. (1981) X-ray crystal structure analysis of 14-hydroxycaryophyllene oxide, a new metabolite of (-)-caryophyllene, in rabbits. *J. Pharm. Sci.*, **70**(6), 710-711
- Budavari, S., ed. (1996) *The Merck Index*, 12th ed., Whitehouse Station, NJ, Merck & Co., Inc., p. 308
- Elias, V.O., Simoneit, B.R.T. & Cardoso, J.N. (1997) Analysis of volatile sesquiterpenoids in environmental and geological samples. *J. High Resolut. Chromatogr.*, **20**(6), 305-309
- FDA (1994) *Priority-based Assessment of Food Additives (PAFA) Database*, Center for Food Safety and Applied Nutrition, US Food and Drug Administration
- FEMA (1997) *FEMA Database: beta-Caryophyllene (FEMA No. 2252)*, Washington, DC, Flavor and Extract Manufacturers Association, 12 pp.
- Fluka Chemical Corp. (1997) *Fluka Chemika BioChemika Analytica*, Milwaukee, WI, p. 338
- Guenther, A., Zimmerman, P. & Wildermuth, M. (1994) Natural volatile organic compound emission rate estimates for U.S. woodland landscapes. *Atmospheric Environ.*, **28**(6), 1197-1210
- Heck, J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B. & Curren, R.D. (1989) An evaluation of food flavoring ingredients in a genetic toxicity screening battery. *Toxicologist*, **9**(1), 257
- Kim, J.O., Kim, Y.S., Lee, J.H., Kim, M.N., Rhee, S.H., Moon, S.H. & Park, K.Y. (1992) Antimutagenic effect of the major volatile compounds identified from mugwort (*Artemisia asiatica nakai*) leaves. *J. Korean Soc. Food Nutr.*, **21**(3), 308-313
- Knöppel, H. & Schauenburg, H. (1989) Screening of household products for the emission of volatile organic compounds. *Environ. Int.*, **15**(1-6), 413-418
- Kubo, I., Chaudhuri, S.K., Kubo, Y., Sanchez, Y., Ogura, T., Saito, T., Ishikawa, H. & Haraguchi, H. (1996) Cytotoxic and antioxidative sesquiterpenoids from *Heterotheca inuloides*. *Planta Med.*, **62**(5), 427-430

- LaVoie, E.J., Adams, J.D., Reinhart, J., Rivenson, A. & Hoffmann, D. (1986) Toxicity studies on clove cigarette smoke and constituents of clove: determination of the LD<sub>50</sub> of eugenol by intratracheal instillation in rats and hamsters. *Arch. Toxicol.*, **59**(2), 78-81
- Lide, D.R., ed. (1995) *CRC Handbook of Chemistry and Physics*, 76th ed., Boca Raton, FL, CRC Press, p. 3-84
- Martin, S., Padilla, E., Ocete, M.A., Galvez, J., Jimenez, J. & Zaruelo, A. (1993) Anti-inflammatory activity of the essential oil of *Bupleurum fruticosens*. *Planta Med.*, **59**(6), 533-536
- NLM (1997) *RTECS (Registry of Toxic Effects of Chemical Substances)*, Bethesda, MD, National Library of Medicine, searched September, 1997 [Record No. 20791]
- Opdyke, D.L.J. (1973) Monographs on fragrance raw materials: Caryophyllene. *Food Cosmet. Toxicol.*, **11**, 1059-1060
- Polarome International (1997) *Material Safety Data Sheet: Caryophyllene*, Jersey City, NJ, 2 pp.
- Ross, S.A. & ElSohly, M.A. (1996) The volatile oil composition of fresh and air-dried buds of *Cannabis sativa*. *J. Nat. Prod.*, **59**(1), 49-51
- Sasaki, Y.F., Imanishi, H., Ohta, T. & Shirasu, Y. (1989) Modifying effects of components of plant essence on the induction of sister-chromatid exchanges in Chinese hamster ovary cells. *Mutat. Res.*, **226**, 103-110
- Sigma (1997) *Biochemicals and Reagents for Life Science Research*, Sigma Chemical Co., St. Louis, MO, p. 239
- Tambe, Y., Tsujiuchi, H., Honda, G., Ikeshiro, Y. & Tanaka, S. (1996) Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene,  $\beta$ -caryophyllene. *Planta Med.*, **62**(5), 469-470
- TCI America (1996) *TCI America 96/97 Catalog*, Portland, OR, p. 297
- US International Trade Commission (1984) *Synthetic Organic Chemicals, US Production and Sales, 1983 (USITC Publication 1588)*, Washington, DC, US Government Printing Office, p. 126
- US International Trade Commission (1985) *Synthetic Organic Chemicals, US Production and Sales, 1984 (USITC Publication 1745)*, Washington, DC, US Government Printing Office, pp. 124, 127
- US International Trade Commission (1986) *Synthetic Organic Chemicals, US Production and Sales, 1985 (USITC Publication 1892)*, Washington, DC, US Government Printing Office, pp. 126, 129

US International Trade Commission (1987) *Synthetic Organic Chemicals, US Production and Sales, 1986 (USITC Publication 2009)*, Washington, DC, US Government Printing Office, pp. 98, 100

US International Trade Commission (1988) *Synthetic Organic Chemicals, US Production and Sales, 1987 (USITC Publication 2118)*, Washington, DC, US Government Printing Office, p. 7-6

US International Trade Commission (1989) *Synthetic Organic Chemicals, US Production and Sales, 1988 (USITC Publication 2219)*, Washington, DC, US Government Printing Office, p. 7-6

US International Trade Commission (1990) *Synthetic Organic Chemicals, US Production and Sales, 1989 (USITC Publication 2338)*, Washington, DC, US Government Printing Office, p. 7-6

US International Trade Commission (1991) *Synthetic Organic Chemicals, US Production and Sales, 1990 (USITC Publication 2470)*, Washington, DC, US Government Printing Office, p. 7-5

US International Trade Commission (1993) *Synthetic Organic Chemicals, US Production and Sales, 1991 (USITC Publication 2607)*, Washington, DC, US Government Printing Office, p. 7-5

US International Trade Commission (1994a) *Synthetic Organic Chemicals, US Production and Sales, 1992 (USITC Publication 2720)*, Washington, DC, US Government Printing Office, p. 3-17

US International Trade Commission (1994b) *Synthetic Organic Chemicals, US Production and Sales, 1993 (USITC Publication 2810)*, Washington, DC, US Government Printing Office, pp. 316-317

Van, H., ed. (1995) *OPD 1996 Chemical Buyers Directory*, 82nd ed., New York, Schnell Publishing Co., p. 200

Wilkins, C.K. & Larsen, K. (1997) Identification of volatile (micro) biological compounds from household waste and building materials by thermal desorption-capillary gas chromatography-mass spectroscopy. *J. High Resolut. Chromatog.*, **18**(6), 373-377

Wise, M.B. & Guerin, M.R. (1986) Chemical analysis of the major constituents in clove cigarette smoke. In: Hoffmann, D. & Harris, C.C., eds., *Banbury Report, 23. Mechanisms in Tobacco Carcinogenesis, Conference Sept. 1985*, Cold Spring Harbor, NY, pp. 151-162

Zheng, G.Q., Kenney, P.M. & Lam, L.K.T. (1992) Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. *J. Nat. Prod.*, **55**(7), 999-1003