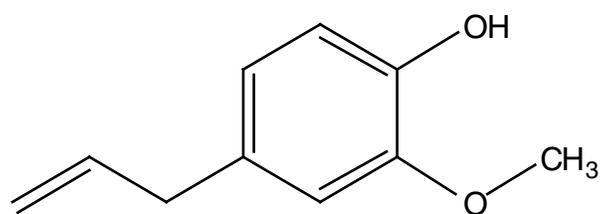


## Chapter 6—Clove Oil (Eugenol)

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Eugenol

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## 6.1 Introduction

Clove oil is an essential oil from the dried flower buds, leaves and stems of the tree *Syzygium aromaticum* (Eastern Hemisphere) or *Eugenia caryophyllata* and *Eugenia aromaticum* (Western Hemisphere).<sup>1</sup> There are only small differences between these species and many consider them to be essentially the same.

When applied to growing plants in sufficient quantities, clove oil rapidly desiccates green tissue by removing the waxy cuticle of the plant and disrupting the cell membrane. This results in electrolyte leakage from plant cells, causing tissue death. Clove oil is not translocated in treated plants and provides no residual weed control.<sup>2</sup> It is only effective as a post-emergent herbicide and provides burndown of both annual and perennial broadleaf and grass weeds. It is also used as an insecticide and as a scent attractant in traps for Japanese beetles, wasps, and other insects.<sup>3</sup>

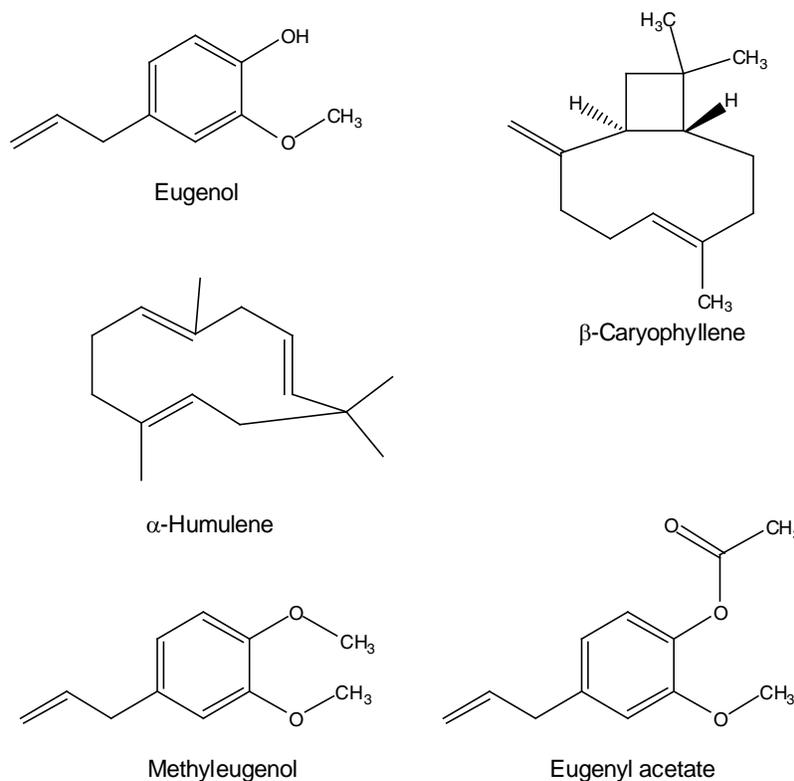
Clove oil is a naturally occurring food flavour and is extensively used in fragrance and flavor formulations for its spicy aroma. Clove oil and its primary ingredient eugenol have been in widespread use as flavoring and fragrance agents in the United States since before 1900. The soap and detergent industry is a major user of both materials, and eugenol is typically used in such products at concentrations in the range of 0.05–0.1% (v/v). The Environmental Working Group's cosmetics database lists 278 cosmetic and personal care products available over-the-counter that contain eugenol in low concentrations.<sup>4</sup>

The US Food and Drug Administration (FDA) has approved clove oil for use in food as a flavoring agent, in dentistry as an analgesic and in dental cements, as a fragrance in personal care products and in aromatherapy oils, and in transdermal drug delivery systems.<sup>5</sup> Clove oil has also been found to possess antibacterial, antifungal, antiviral, antitumor, antioxidant and insecticidal properties.<sup>6</sup> The FDA categorizes clove oil as generally recognized as safe (GRAS) for use in dental cement or as a food additive, but neither clove oil nor any of its components are rated as GRAS for use as an anesthetic for fish because of concerns about toxicity to fish and to humans consuming fish treated with clove oil.<sup>7</sup>

The US EPA has placed clove oil on the Section 25(b) list of Minimum Risk Pesticides. Pesticides on this list are exempt from most pesticide registration requirements, including extensive toxicity testing; thus, no Reregistration Eligibility Decision document has been issued for clove oil.<sup>8</sup> Clove oil was first registered as a pesticide in 1972 and reregistered in 1993. Herbicide products containing clove oil as the primary active ingredient have only become available in the last few years. Products containing only Section 25(b) active ingredients and minimal risk other ingredients on List 4A are exempt from registration and are not issued a registration number. Because pesticide use in California is tracked by product registration number, no use statistics are available for exempt clove oil-containing pesticide products.

Clove oil is comprised of many different compounds, with the primary ingredients being eugenol (49–87%),  $\beta$ -caryophyllene (4–21%), and eugenyl acetate (0.5–21%). Smaller amounts of  $\alpha$ -humulene are also present, as well as trace amounts (<1%) of 25–35 other constituents. Figure 6-1 shows the chemical structures of the primary components of clove oil. Several factors govern the relative quantities of the different constituents in clove oil, including plant genetics, climate,

soil and cultivation techniques, the part of the plant extracted, and the extraction method.<sup>9</sup> Table 6-1 summarizes the composition of clove oil from available studies.



**Figure 6-1:** Primary chemical components of clove oil.

Interestingly, both FDA<sup>7</sup> and EPA<sup>10</sup> indicate that clove oil contains 5–15% methyl eugenol, but none of the studies of clove oil composition support this statement (Table 6-1). This is an important distinction, since the US National Toxicology Program (NTP) determined that methyl eugenol is “Reasonably Anticipated to be a Human Carcinogen” because of its carcinogenicity in rodents.<sup>11</sup> The NTP also evaluated eugenol and found evidence of carcinogenicity in mice but not rats. The data were equivocal and not sufficient to prompt a listing of eugenol as a carcinogen.

More important for the MMWD project is that Matran, the product under consideration for use in the vegetation management program, contains only eugenol as the active ingredient. The clove leaf oil used in Matran is distilled to separate eugenol from the other constituents.<sup>12</sup> Matran is 50 percent eugenol; the remaining 50 percent is comprised of wintergreen oil, butyl lactate, and lecithin, all of which are classified by EPA as minimal risk other ingredients.

This chapter focuses on the human toxicity, ecotoxicity, and environmental fate of clove oil extracts and eugenol, drawing primarily from the World Health Organization JEFCA reports on eugenol,<sup>13, 14</sup> the US National Toxicology Program (NTP) review of the carcinogenicity of eugenol,<sup>15</sup> and the peer-reviewed literature for clove oil and eugenol.

**Table 6-1: The Composition of Clove Oil from Different Sources**

Clove Oil Source	Extraction Method <sup>a</sup>	Eugenol (%)	Eugenyl Acetate (%)	$\beta$ -Caryophyllene (%)	Methyl Eugenol (%)	Other Primary Constituents	Reference
Leaves	NR	76.8	1.2	17.4	TR	$\alpha$ -Humulene, 2.1% All others < 1%	16
Buds <sup>b</sup>	SFE	55.41	21.06	16.22	ND	$\alpha$ -Humulene, 2.11% All others < 1%	17
Buds	SD	58.2	13.84	20.59	ND	$\alpha$ -Humulene, 2.61% Cadenine, 1.05% All others < 1%	17
Buds	HD	48.82	3.89	36.94	ND	$\alpha$ -Humulene, 4.41% Cadenine, 1.46% All others < 1%	17
Buds	SO	57.24	19.37	17.5	ND	$\alpha$ -Humulene, 2.03% All others < 1%	17
Buds-Turkey	SD	87.00	8.01	3.56	< 0.01	All < 1% $\alpha$ -Humulene, 1.9% $\alpha$ -Bergotamine, 1.3%	9
Buds-India	HD	70.0	2.1	19.5	ND	All others < 1% $\alpha$ -Humulene, 0.8%	18
Buds-Madagascar	HD	82.6	6.0	7.2	< 0.05	All others < 1% $\alpha$ -Humulene, 1.5%	18
Leaves-Madagascar	HD	82.0	0.4	13.0	ND	All others < 1%	18
Buds	SD	89.27	8.62	ND	ND	All < 1%	19
NR	HD	88.58	5.62	1.39	< 0.1	All < 1% $\alpha$ -Humulene, 0.36%	6
Leaves-India	HD	94.41	ND	2.91	NR	All others < 1% $\alpha$ -Humulene, 1.75%	20
Leaves-Indonesia	NR	71.0	NR	14.0	NR	All others NR	21
Leaves-India	NR	95.2	1.5	TR	NR	NR	22
Sigma-Aldrich	NR	78	NR	13	NR	NR	23

TR = trace. ND = not detected. NR = not reported.

<sup>a</sup> NR = not reported; SD = steam distillation; SFE = supercritical fluid extraction with CO<sub>2</sub>; HD = hydrodistillation; SO = soxhlet extraction.

<sup>b</sup> Average of nine different SFE extractions.

## 6.2 Clove Oil and Eugenol Toxicity to Humans and Levels of Concern

There are no studies of acute or chronic adverse effects from occupational exposure to clove leaf oil or eugenol when used as a pesticide. Eugenol is not acutely toxic, with an oral LD<sub>50</sub> of 2,650 mg/kg in the rat. It is a mild skin and eye irritant (Category III). Clove oil and eugenol are classified by EPA as minimum risk pesticides, and products containing them are exempt from the requirements of FIFRA. The state of California requires that exempt pesticide products containing 8.5 percent or more of clove oil include the signal word “CAUTION” and the phrase “Keep Out of Reach of Children,” appropriate precautionary language, and a requirement for protective eyewear and gloves on the label. Clove oil is rapidly absorbed through the skin and is used in patented systems for dermal drug delivery to enhance drug uptake from skin patch delivery systems.

There are no epidemiological studies of the effects of clove oil in humans. There are several case reports of acute toxic effects of clove oil exposure; The California Pesticide Incident Surveillance Program (PISP) reported only two cases over a ten year period.

### **6.2.1 Health Effects**

Clove oil is considered safe in small quantities (< 1,500 ppm) as a food additive. However, clove oil is toxic to human cells. If ingested in sufficient quantity or injected, it has been shown to cause life-threatening complications, including Acute Respiratory Distress Syndrome, Fulminant Hepatic (Liver) Failure, and Central Nervous System Depression; the lethal oral dose is 3.75 g per kg body weight.

There are no epidemiological studies of potential adverse human health effects related to exposure to clove leaf oil or eugenol from any human exposure scenarios. Nor are there any studies of agricultural use, either in workers or those with bystander exposures from drift or other applications. There are no occupational exposure standards for clove leaf oil or eugenol including OSHA PEL (Permissible Exposure Limit) or AGIHA TLVs (Threshold Limit Value) in air.

#### ***6.2.1.A Acute Effects—Sensitization***

Traditional uses of clove leaf oil include treating burns and cuts, and it has also found use in dental care as a pain reliever, and undiluted clove oil may be rubbed on the gums for treating tooth infections and toothache. There are studies from several decades ago that show eugenol to be a contact allergen when used in dentistry.<sup>24, 25</sup>

Although clove oil and eugenol are known sensitizers (cause allergic skin reactions) in experimental animals, results from 11,632 patch tests of various consumer products and fragrance blends collected from fragrance and formulation companies found a very low potential to elicit pre-existing sensitization or to induce hypersensitivity. One instance of induced hypersensitivity and one instance of pre-existing sensitization were observed at eugenol patch-test concentrations of  $5 \times 10^{-2}$  and  $9 \times 10^{-2}$  percent.<sup>26</sup>

Clove oil was also found to be a mosquito repellent when 0.1 mL was applied per 30 cm<sup>2</sup> of exposed skin of human volunteers.<sup>27</sup>

#### ***6.2.1.B Acute Effects—Skin, Eyes and Respiratory System***

Matran is corrosive, and label warnings state that contact can cause severe eye irritation with possible permanent damage, severe skin irritation and/or chemical burns, significant respiratory irritation, and pulmonary edema from prolonged inhalation, and that ingestion could cause burns and destroy tissue in the mouth, throat, and digestive tract.

#### ***6.2.1.C Acute Effects—Systemic Poisoning***

Clove oil is toxic to the liver and nervous system. In all case reports of acute illness or death, it was ingested.<sup>28, 29</sup> A two year-old child suffered hepatocellular necrosis following ingestion of clove oil.<sup>30</sup> A three month-old infant developed fulminant hepatic failure after ingesting less than 8 mL of clove oil.<sup>31</sup> A fifteen month-old boy developed fulminant hepatic failure after ingesting

10 ml of clove oil in an aromatherapy product.<sup>32</sup> A 7-month-old child developed central nervous system depression after the accidental oral administration of clove oil.<sup>33</sup>

A 32 year old woman developed severe dyspnea (difficulty breathing) and pulmonary edema (fluid in the lungs) after injecting clove oil intravenously.<sup>34</sup> Smoking clove cigarettes (60-70 percent tobacco, 30-40 percent cloves) has been reported to cause respiratory effects including bronchospasm, haemoptysis (spitting up blood), and pulmonary edema.<sup>35</sup>

A five percent solution of a eugenol derivative studied for use as an intravenously anesthetic in 100 adults undergoing surgery produced a transient respiratory stimulation followed by a brief period of apnea. Thirty-three patients developed venous thrombosis around the site of injection; this incidence is too high to justify its use except in special circumstances.<sup>36</sup>

#### **6.2.1.D Effects in Human Cells**

Eugenol was found to be cytotoxic to the human osteoblastic cell line U2OS in an *in vitro* study. The inhibition of growth and proliferation was dose-dependent.<sup>37</sup> Clove oil was highly cytotoxic to human skin cells at concentrations as low as 0.03 percent (v/v) with the effect attributable to eugenol.<sup>38</sup> This level is much lower than the concentrations found in consumer products containing eugenol sold over-the-counter.

Clove oil and eugenol were found to be spermicidal in an *in vitro* study of six male partners of infertile couples.<sup>39</sup>

Polymorphonuclear leukocytes (a type of white blood cell) release inflammatory mediators such as leukotrienes which are implicated in allergic and inflammatory disorders such as asthma, allergic rhinitis, arthritis, inflammatory bowel disease and psoriasis. The activity of a key enzyme in the biosynthetic pathway of leukotrienes—5-lipoxygenase (5-LO)—is inhibited by eugenol, which may have beneficial role in modulating leukotriene release in humans.<sup>40</sup>

Clove oil has also been shown to have an anti-thrombotic (decreases blood clotting) effect in humans, due to its inhibition of platelet aggregation and thromboxane synthesis.<sup>41</sup>

Eugenol is contained in various endodontic medications and applied directly to the teeth. Widely used in dentistry, eugenol penetrates the dental pulp tissue and can enter the bloodstream. A recent study found that eugenol induced chromosome aberrations in human dental pulp cells, and suggested it was a potential mutagen.<sup>42</sup>

A recent short review addresses the chemical composition and biological effects of clove essential oil, and includes new results from GC/MS analysis and a study of its antimicrobial activity against a large number of multi-drug resistant *Staphylococcus epidermidis* bacteria isolated from dialysis biomaterials.<sup>6</sup>

#### **6.2.1.E Levels of Concern for Humans**

Because eugenol is exempt from most EPA registration requirements because it is a Section 25(b) minimum risk pesticide, EPA has not developed an RfD. The WHO developed an Acceptable Dietary Intake (ADI) value for eugenol of 2.5 mg/kg-day, based on a rat study with a

NOAEL-equivalent of 250 mg/kg-day. This value used as the RfD for the MMWD risk assessment.

### 6.2.2 Pesticide Illness Reports

The California Pesticide Illness Surveillance Program (PISP) reported only two clove oil-related poisonings, one each in 2001 and 2002 (see Table 6-2). There were no fatalities.<sup>43</sup>

**Table 6-2: Clove Oil-related Illnesses Reported by California Physicians to the Pesticide Incident Surveillance Program (PISP), 2001 and 2002**

Year	Total Reported Pesticide Cases		Clove Oil Cases				Total (%)
	Definite/Probable	Possible	Systemic/Respiratory		Local/Topical		
	Definite/Probable	Possible	Definite/Probable	Possible	Definite/Probable	Possible	
2002	1,025	291	1	0	0	0	1 (0.09)
2001	430	186	1	0	0	0	1 (0.23)

**Toxic Exposure Surveillance System (TESS):** Table 6-3 summarizes clove oil-related TESS data from 2003 to 2006. The data represent contact with a poison control center, usually by telephone, regarding a potential poisoning problem, and does not mean that poisoning or illness definitely occurred. This is reflected in the combined percentage of ‘None’ and ‘Minor’ adverse outcomes shown in the table. For clove oil, major outcomes are those related to accidental and suicidal ingestion. Outcomes are not known for all calls to the center, and the data in the table are based on cases for which follow up information was known, usually because of treatment in a medical care facility.

**Table 6-3: Calls to U.S. Poison Control Centers Related to Clove Oil Exposure 2003 to 2006<sup>44</sup>**

Year	Total Cases	Age			Causation			Adverse Outcome					
		< 6	6-19	>19	Uninten-tional	Inten-tional	Other	None	Minor	None + Minor	Mod	Major	Death
2006	373	245	33	80	346	10	3	71	100	(46%)	7	0	0
2005	446	277	34	133	416	6	1	109	10	(26%)	7	0	0
2004	444	277	26	137	420	9	0	103	106	(47%)	8	0	0
2003	870	645	52	172	826	13	3	208	171	(44%)	12	1	0

\* Source: <http://www.aapcc.org/dnn/NPDS/AnnualReports/tabid/125/Default.aspx>

### **6.3 Eugenol Toxicity to Animals and Plants and Levels of Concern**

This section summarizes the toxicity of clove oil or its primary component eugenol to seven taxa groups, including mammals, fish, amphibians, terrestrial and aquatic invertebrates, terrestrial plants, and microorganisms. Clove oil is exempt from many EPA registration requirements and is classified as GRAS for most purposes by the FDA. Because of the exemption from registration requirements, few toxicological studies are available from EPA, but NTP and WHO have both evaluated eugenol toxicity. There are many studies on clove oil and its chemical components in the academic literature, but determination of toxicological endpoints is often not the focus of these studies.

Levels of concern for clove oil are also summarized in this section, with Table 6-8 on page presenting the toxicity reference values (TRVs) selected for the MMWD risk assessment.

Acute and chronic clove oil toxicity to mammals is low. Acute oral LD<sub>50</sub> values in all species tested were greater than 1,190 mg/kg. In subchronic toxicity tests, no adverse effects were observed in studies with laboratory animals up to doses of 900 mg/kg-day. At higher doses, liver damage has been observed. There is some equivocal evidence for carcinogenicity, but not sufficient for a listing as a carcinogen.

Clove oil toxicity to birds and aquatic plants is not available; only one study, with no toxicology endpoint, is available for amphibians. Clove oil toxicity to insects is highly variable, with some insect orders being quite sensitive and others being quite tolerant. Clove oil is highly toxic to microbes, altering the cell membranes of yeasts and bacteria. At application rates that are somewhat higher than conventional herbicides, clove oil is toxic to terrestrial plants. Clove oil is classified as moderately to not acutely toxic to fish and is even used as an anesthetic for fish at low doses. Exposure duration accounts for some of the wide variability in clove oil toxicity to fish.

Only studies with LD<sub>50</sub> or LC<sub>50</sub> endpoints were available for the ecotoxicity data; thus, the TRVs were adjusted downward to more accurately represent a no-effect level. This approach uses EPA methodology for assessing effects on endangered species.<sup>45</sup> The adjustment employed was to divide the LC<sub>50</sub> by six (or 20 in the case of salmonids), based on an extensive review of existing ecotoxicological data on pesticides.<sup>46</sup> The review noted that sublethal effects did not typically occur at concentrations below one-fourth to one-sixth of the LD<sub>50</sub>, when taking into account the same percentages or numbers affected, test system, duration, species, and other factors. This effect is termed the “6x hypothesis.” However, it should be noted that this review is almost 30 years out-of-date, and that the factor of six is meant to translate an LC<sub>50</sub> to a NOEC *of the same species*. The use of a single NOEC for all species in a taxa group suggests that interspecies variability may not be fully accounted for by the factor of six. Further, the factor of six appears to be too low for salmonids. As discussed in the EPA report, salmonids’ olfactory ability seems to be particularly sensitive to pesticide concentrations 20 times lower than the LC<sub>50</sub>.<sup>47</sup> Thus, for fish, the LC<sub>50</sub> values are divided by 20 to obtain the TRV used in the MMWD risk assessment.

### 6.3.1 Mammals

Comprehensive summaries of data on the toxicity of eugenol to mammals are contained in the documents produced by WHO<sup>13, 48</sup> and NTP.<sup>15</sup> Much of this research is peer-reviewed and available in the scientific literature. Results of toxicity studies in laboratory animals are summarized in Tables 6-2 to 6-4.

Eugenol is rapidly absorbed and metabolized in the liver when ingested, and 95% of the dose is excreted within 24 hours. Acute toxicity is low by the oral route, with LD<sub>50</sub> values ranging from 1,190–3,000 mg/kg-day. High oral doses of eugenol are acutely toxic to the liver in dogs and rats. Eugenol is a mild to moderate eye and skin irritant depending on the formulation.

Subchronic toxicity is low, with NOAELs ranging from 900–>2,000 mg/kg-day. No data are available on endocrine disruption, reproductive, developmental, neurological, and immunotoxicity.

The EPA has not evaluated the carcinogenicity of eugenol, but NTP did in 1983.<sup>15</sup> Eugenol was not carcinogenic in a lifetime study in the rat; however, a lifetime study with mice showed evidence of an increased incidence of liver tumours. Statistical analysis of the results suggested a positive trend in male mice. Although the incidence of tumours in the female mice was increased at the low dose level, the effect was not dose-related. Eugenol gave both positive and negative results in tests for DNA damage in bacteria. It was not mutagenic in several studies in bacteria, but chromosome aberrations were observed in mammalian tissue cultures. The significance of these mixed results is not clear, and the US National Toxicology Program determined that the data were not sufficiently robust to prompt a listing of eugenol as a carcinogen. The International Agency for Research on Cancer (IARC) lists eugenol as class 3, Unclassifiable.

#### 6.3.1.A Metabolism and Pharmacokinetics of Eugenol

Eugenol is rapidly absorbed in the digestive tract and metabolized in the liver primarily to the glucuronic acid or sulfate conjugate (see Figure 6-2).<sup>14</sup> In humans, 95% of ingested eugenol is excreted in conjugated form in the urine within 24 hours, with the sulfate conjugates predominating at low doses and the glucuronic acid conjugates at higher doses (>500 mg/kg-day). Concentrations of eugenol in blood and plasma peak rapidly following oral ingestion, but mean half-lives for eugenol in plasma and blood were 14.0 and 18.3 h, respectively.<sup>49</sup>

Minor metabolic pathways include oxidation of the side-chain double bond to the epoxide, followed by hydrolysis to the diol and further oxidation; isomerization to form isoeugenol, followed by allylic oxidation and then reduction; conjugation of an oxidation intermediate with glutathione; and reduction of the side-chain double bond (see Figure 6-2). All metabolites have an aromatic hydroxyl group that reacts readily with glucuronic acid or sulfate to form the conjugates, which are readily excreted in the urine. Rodent metabolism is similar to that in humans. A number of studies have been done to elaborate the various metabolic pathways; these are well-described in reference 14.

The various enzymes responsible for the metabolism of eugenol function efficiently up to doses of approximately 400–600 mg/kg in rats and mice and in some cases higher; above these doses,

these metabolic pathways start to become saturated and liver toxicity is observed. A toxic response may also result when there is simultaneous exposure to eugenol and another chemical that utilizes the same metabolic pathways or in the presence of another chemical that inhibits the enzymes necessary for the degradation of eugenol. Eugenol also has been found to decrease DNA damage from known carcinogens by inhibiting the enzymes responsible for adduct formation with electrophilic substrates and by induction of the enzymes responsible for detoxification.<sup>50</sup>

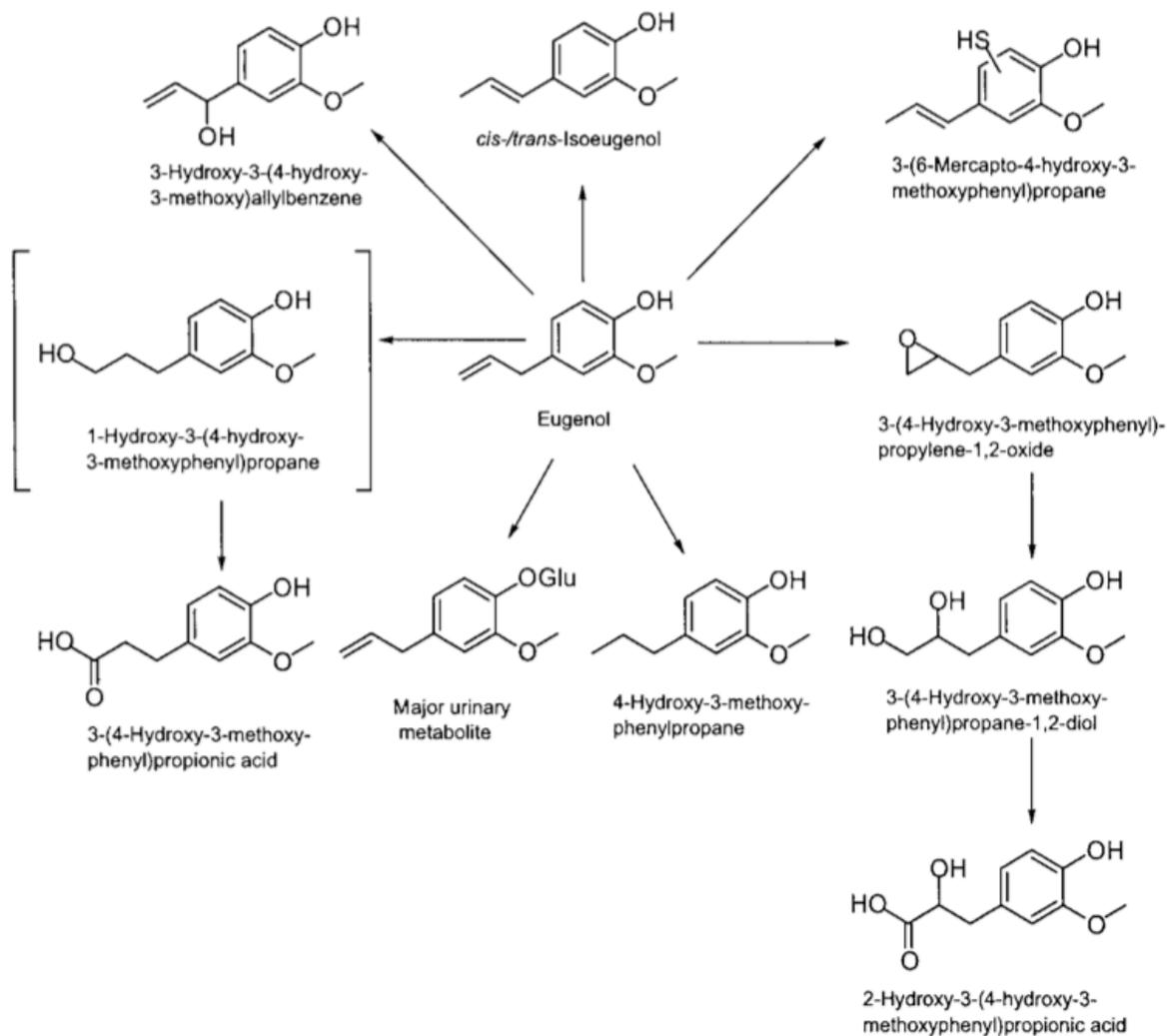


Figure 6-2: Metabolic pathways of eugenol in humans. Abstracted from reference 14.

### 6.3.1.B Acute Toxicity of Eugenol

The acute oral, dermal and inhalation toxicity in mammals of eugenol is low (see Table 6-4). Acute toxic effects at high doses include destruction of the gastric mucosa,<sup>51</sup> capillary hemorrhaging in dogs,<sup>52</sup> gastric inflammation and depression of secretory capacity,<sup>53</sup> liver discoloration and mottling in rats,<sup>54</sup> and liver congestion in dogs.<sup>55</sup>

**Dermal and ocular exposure:** No dermal or ocular exposure toxicity tests are available, but eugenol has been patented as an enhancement agent in transdermal drug delivery systems,<sup>5</sup>

which indicates that eugenol is readily absorbed through the skin. Products containing eugenol or clove oil may cause irritation to the skin and eyes.

**Inhalation exposure:** The vapor pressure of eugenol is moderately high (0.0226 mm Hg at 25°C), indicating that inhalation may contribute substantially to exposure, especially for workers. No inhalation toxicity studies were available.

**Table 6-4: Acute Toxicity of Eugenol and Clove Oil in Experimental Animals**

Mode	Formulation	LD <sub>50</sub> (mg/kg)	EPA Toxicity Category
Rat oral <sup>53</sup>	Eugenol	1,930	III
Rat oral <sup>54</sup>	Eugenol	2,680	III
Rat oral <sup>56</sup>	Eugenol	1,190	III
Mouse, oral <sup>57</sup>	Eugenol	3,000	III
Mouse, intraperitoneal <sup>58</sup>	Eugenol	500	II
Mouse, intraperitoneal <sup>59</sup>	Eugenol	630	III
Guinea pig, oral <sup>57</sup>	Eugenol	2,130	III

**Anesthetic effects:** Intravenously administered eugenol has been found to produce reversible, dose-dependent anesthetic effects in rats at moderate doses (5–60 mg/kg) given intravenously.<sup>60</sup>

**Cardiovascular effects:** Eugenol has vasorelaxant properties. The relaxant effects of eugenol at 300 micromoles per liter (μM) were comparable to those induced by nifedipine, a selective Ca<sup>2+</sup> channel blocker, at 0.01 μM, producing similar relaxant effects.<sup>61</sup> The authors concluded that eugenol produces smooth muscle relaxation resulting from the blockade of both voltagesensitive and receptor-operated channels that are modulated by endothelial-generated nitric oxide.

A second study evaluated the effects of injections of 1–10 mg eugenol/kg on vascular relaxation. Dose-dependent hypotension and bradycardia were observed.<sup>62</sup> The authors indicate that “The bradycardia appears dependent upon the presence of an intact and functional parasympathetic nerve drive to the heart while the hypotension is due to an active vascular relaxation rather than withdrawal of sympathetic tone.” These authors concluded that nitric oxide from vascular endothelial cells was *not* involved in the mediation of eugenol-induced hypotension.

**Anti-fever effects:** Eugenol was found to reduce fever in rabbits when given intravenously in low doses.<sup>63</sup> Eugenol was more effective in reducing fever than acetaminophen.

### 6.3.1.C Subchronic Toxicity of Eugenol

The subchronic toxicity of eugenol is low, with most studies showing no effects until a very high dose regime is entered. Up to 1.0% eugenol in the diet did not cause adverse effects. At intermediate doses by gavage, reduced weight gains, erosion of the epithelium in the stomach, and liver damage were observed. At the highest doses (10–12% of the diet), most of the test animals died. The available studies are summarized in Table 6-5.

Single-dose studies in rats were used to determine the level at which no toxic effects were observed, and 250 mg/kg was selected as the NOAEL-equivalent by WHO. This dose was used to develop the acceptable daily intake (ADI) of 2.5 mg/kg-day for humans, dividing the NOAEL by the intra- and inter-species factors of 10.

**Table 6-5: Subchronic Toxicity of Eugenol**

Test animal	Study Duration (days)	Doses Tested (mg/kg-day)	Dose (endpoint) (mg/kg-day)	Observed Effects
Rats, 10 M, 10 F <sup>64</sup>	90	89.7	>89.7	No adverse effects observed.
Rats, 5 M <sup>65</sup>	28	NR	>2,000	NR
Rats, 20 M <sup>66</sup>	34	1,400–4,000, gavage (increasing doses over the time period)	<1,400	Considerable mortality was observed; also slight liver enlargement and adrenal enlargement. Histology showed enlarged liver cells. The forestomach showed moderately severe hyperplasia and hyperkeratosis of the stratified squamous epithelium with focal ulceration.
Rats, 10 M, 10 F <sup>67</sup>	133	0.1 and 1.0% in the diet	>1,000	No adverse effects on growth rate, hematology, organ weights and histology of major tissues.
Rats, F-344 5 M, 5 F <sup>68</sup>	14	0.6, 1.25, 2.5, 5 or 10% in the diet (6,000, 12 500, 25,000, 50,000 or 100,000 ppm in the diet). No concurrent controls.	NR	One high dose male and all high dose females died during the study. There appeared to be a dose-related reduction in weight gain.
Rats, F-344 10 M, 10 F <sup>68</sup>	90	0.08, 0.15, 0.3, 0.6 or 1.25% in the diet (800, 1,500, 3,000, 6,000 or 12,500 ppm in the diet)	>1,250	No compound-related effects reported. No mortality or gross or microscopic pathology. Relative to controls, weight gain was reduced 12% in the high dose males.
Mice, B6C3F1 5 F, 5 M <sup>68</sup>	14	0.6, 1.25, 2.5, 5 or 10% in the diet (6,000, 12 500, 25,000, 50,000 or 100,000 ppm in the diet). No concurrent controls.	NR	Dose-related decrease in weight gain in both males and females. All 5 of the males in the 100 000 ppm (10%) group died before the end of the study. In the females, all of the 100 000 ppm (10%) group died before the end of the study.
Mice, B6C3F1 10 M, 10 F <sup>68</sup>	91	0, 0.04, 0.08, 0.15, 0.3 or 0.6% in the diet (400, 800, 1,500, 3,000 or 6,000 ppm in the diet).	>900	No mortality or compound-related gross or microscopic pathology
Mice, 114 unspec. <sup>69</sup>	35 dosing days 420 total days	410 2x/wk, gavage	410	NR
Mice, 30 unspec <sup>69</sup>	365	NR, diet	>750	NR

M = male; F = female; NR = not reported

#### **6.3.1.D Chronic Toxicity and Carcinogenicity of Eugenol**

The carcinogenicity of eugenol has been the subject of several reviews by WHO's Joint FAO/WHO Expert Committee on Food Additives (JECFA) and NTP. The International Agency for Research on Cancer (IARC) ranks eugenol as Unclassifiable for carcinogenicity, based on the equivocal results obtained in animal studies.<sup>70</sup> Eugenol was not carcinogenic to rats; in mice, significant increases in liver tumors were observed. Eugenol gave both positive and negative results in mutagenesis tests, induced chromosomal aberrations and increased sister chromatid exchanges in *in vitro* tests. Based on the limited and conflicting evidence of carcinogenicity, neither IARC nor NTP listed eugenol as a carcinogen. The chronic animal studies are summarized in Table 6-6.

Eugenol was reported to inhibit the carcinogenicity of benzo(a)pyrene when the compounds were applied together in a carcinogenic skin-painting study.<sup>71</sup> In a limited study in mice, eugenol did not potentiate the tumorigenic effects of methylcholanthrene.<sup>72</sup>

The mutagenic and chromosomal effects of eugenol have also been investigated in *in vitro* studies. Findings are contradictory, reflecting the results of the *in vivo* studies. Mutagenesis assays using *Salmonella* both with and without activation with the S9 fraction of the liver were negative.<sup>15, 73, 74</sup>

A 2005 assay using Syrian hamster embryo (SHE) cells found that eugenol induces chromosome aberrations.<sup>75</sup> Similar results were observed in V79 cells at higher doses.<sup>76</sup> The S9 fraction of the liver increased the induction of chromosome aberrations in a dose-dependent manner. The results confirm that eugenol is genotoxic and raises the possibility of it having topoisomerase II-inhibiting activity. Topoisomerases are involved in the unwinding of the DNA helix during the replication process.

Eugenol has been found to inhibit the inducible cyclooxygenase (COX2) enzyme that has been implicated in the processes of inflammation and carcinogenesis.<sup>77</sup> Potential COX2 inhibitors have been considered as antiinflammatory or cancer chemopreventive agents.

Eugenol produced a positive recombinagenic response in the somatic mutation and recombination test (SMART) assay using *Drosophila*, which is related to a high cytochrome P450-dependent activation capacity.<sup>78</sup> The authors suggest that this family of enzymes is involved in the activation of eugenol rather than in its detoxification.

**Table 6-6: Chronic Toxicity of Eugenol to Mammals—Cancer**

Test animal	Study Duration (months)	Doses Tested (mg/kg-day)	Dose (endpoint) (mg/kg-day)	Observed Effects
Mice, CD-1 <sup>79</sup> 30 F	12 dosed 6 not dosed	0, 0.5% in diet + 0.05% phenobarbitol, 0.05% phenobarbitol only	NR	In the groups given only phenobarbitol, 3/29 mice developed liver tumours, while no liver tumours were found in the other 3 groups. Only the liver was examined for tumors.
Mice, CD-1 40–60 M, 40–60 F <sup>69</sup>	Dosed for 31 days, starting at 4 days of age. No dosing for 13 months.	0, 2.5 µmol (gavage)	NR	No effects of treatment on the incidence of liver tumours was noted in either sex.
Mice, CD-1 40–50 M <sup>69</sup>		0.63, 1.26, 2.52 and 5.04 µmol (i.p. injection) at days of age of 1, 8, 15, and 22 with eugenol and eugenol epoxide.	NR	As compared to concurrent controls receiving the trioctanoin solvent only, neither treatment group had an increased incidence of liver tumors
Mice, ICR/HA Swiss 20 per dose <sup>80</sup>	63 wks	3x weekly doses Group 1: 0 Group 2: DMBA + 5 mg eugenol Group 3: 5 mg eugenol Group 4: DMBA + DMSO (solvent control)		No carcinomas were found in either group and no papillomas were found in the animals receiving only eugenol. Three animals developed papillomas in the group initiated with DMBA and also treated with eugenol. Two papillomas and 1 carcinoma developed in control animals initiated with DMBA and then treated 3 times weekly with DMSO, the solvent control.
Mice, B6C3F1 50 M, 50 F <sup>15</sup>	103 weeks dosed 2 weeks no dosing	0, 3,000, 6,000 ppm (0, 0.3 and 0.6% in diet)		Small dose-related decrease in weight gain was noted for both males and females throughout the study. No compound-related clinical signs were reported; however, survival was somewhat lower in high dose males and low dose females but not statistically significant. Increased incidence of hepatocellular carcinoma & adenoma in M & F rats. A significant increase in hepatic neoplasms was not observed in high dose animals. No single liver tumor type was observed in female mice with a statistically significant increased incidence. Overall, evidence that eugenol increased liver tumours in B6C3F1 mice; however, the results were judged to be equivocal because of the limited weight of this evidence.

**Table 6-6 (cont): Chronic Toxicity of Eugenol to Mammals—Cancer**

Test animal	Study Duration (months)	Doses Tested (mg/kg-day)	Dose (endpoint) (mg/kg-day)	Observed Effects
Rats, F-344 40–50 M per dose <sup>15</sup>	103 weeks dosed 1–2 weeks no dosing	0, 3,000, 6,000 ppm (M) (0, 0.3 and 0.6% in diet)  0, 6,000, 12,500 ppm (F) (0, 0.6, 1.25%)		Dose-related effects on weight gain (F). Reduction in food consumption. No significant compound-related effects on survival. Increased incidence of endometrial stromal polyps of the uterus (F). Some alveolar-bronchiolar adenomas of the lungs were observed, but not statistically significant. C-cell adenomas of the thyroid gland observed in F rats, statistically significant in low dose animals, but not high-dose animals. There was no increased incidence of this tumour at either dose in the males. Fibroadenomas of the mammary gland were decreased in dosed groups of female rats compared with controls. The conclusion of the report stated that eugenol was not carcinogenic to rats.

NR = not reported; DMBA = 7,12-dimethylbenz(a)anthracene, a mutagen used to initiate a tumorigenic response.

#### **6.3.1.E Reproductive and Developmental Toxicity of Eugenol**

There are no studies available on the reproductive and developmental toxicity of eugenol.

#### **6.3.1.F Neurotoxicity of Eugenol**

There are no studies specifically designed to detect potential adverse effects on the central or peripheral nervous system in mammals exposed to eugenol. One *in vitro* study evaluated the potential of eugenol in the treatment of Parkinson's disease, finding that eugenol prevented the reduction of dopamine and its metabolites, reduced lipid peroxidation, and increased glutathione and L-ascorbate (both antioxidants) in mouse cells.<sup>81</sup> The authors concluded that eugenol may be useful in the treatment of Parkinson's disease.

#### **6.3.1.G Immunotoxicity of Eugenol**

There are no data available to assess the potential immunotoxic effects of eugenol.

#### **6.3.1.H Endocrine Disruption**

There are no studies of eugenol's potential to interact or interfere with estrogen, androgen, thyroid or other endocrine systems. A European Union survey of the scientific literature on endocrine effects of pesticides does not list eugenol or clove oil as a chemical of concern,<sup>82</sup> nor do other sources of information on endocrine disrupting effects.<sup>83</sup> However, no comprehensive evaluation of eugenol or clove oil has been undertaken, and no final conclusions on the endocrine disrupting ability of this compound can be drawn at this time. Eugenol is not one of the first set of chemicals slated for testing of endocrine disrupting effects by the EPA. It is not clear when testing on this herbicide will be done.

#### **6.3.1.I Levels of Concern for Mammals**

The ADI of 2.5 mg/kg-day proposed by WHO for eugenol is based on a rat study with a NOAEL of 250 mg/kg-day. This rat NOAEL is used as the mammalian TRV for the MMWD risk assessment.

### 6.3.2 Other Terrestrial Organisms

This section summarizes information on clove oil or eugenol toxicity to terrestrial animals other than mammals. Table 6-7 provides a summary of available toxicity information and Table 6-8 presents the TRVs used in the risk assessment. Few of the studies provide typical toxicological endpoints, such as an LD<sub>50</sub> or a NOEL. Often, only qualitative endpoints are available. No information is available for clove oil toxicity to birds or honeybees. There are mixed results concerning clove oil toxicity to insects and microorganisms.

#### 6.3.2.A Birds

No studies were available describing clove oil toxicity to birds.

**Levels of concern for birds:** Because there were no bird toxicity studies available, the mammal TRV of 250 mg/kg for acute and chronic exposures was used in the MMWD risk assessment.

#### 6.3.2.B Insects

The insecticidal properties of clove oil have been studied since the 1940s.<sup>84</sup> Clove oil toxicity to different types of insects varies considerably. Very high application rates are needed to effectively control Coleoptera (weevils and beetles), moth caterpillars, lice and cockroaches. Other studies indicate that clove oil and eugenol can be effective at controlling mites, termites and mosquitoes at lower application rates. There were no quantitative studies on honeybees. A summary of available toxicity studies is in Table G-2 in Appendix G.

Because of its importance in pollination, the honeybee is the representative insect used in risk assessments. Although there are multiple studies on the toxicity of clove oil to other insects, there is little information on clove oil toxicity to honeybees. A single study reports that clove oil fed directly to honeybees was considered to be non-toxic.<sup>85</sup> The specifics of the study are unavailable.

Application of clove oil reduces oviposition and adult emergence of the cowpea weevil (*Callosobruchus maculatus*) on a variety of seeds. The exact percent reduction depends on the texture of the seed coat and the type of seed. On bambaranut seeds, oviposition is reduced by roughly 70%.<sup>86</sup> Eugenol also prevents oviposition of the cattle tick, or *Boophilus microplus*, at doses of 1.0 mg/g of seed.<sup>87</sup>

Eugenol, isoeugenol and methyleugenol are not acutely toxic to Coleoptera *Sitophilus zeamais* and *Tribolium castaneum*. For *S. zeamais* all compounds were equally toxic with LD<sub>50</sub> values approximately 30 µg/mg insect (or 30,000 mg/kg). For *T. castaneum*, the order of potency of these chemicals was isoeugenol (LD<sub>50</sub>=21.6 µg/mg insect) > eugenol (LD<sub>50</sub>=30.7 µg/mg insect) > methyleugenol (LD<sub>50</sub>=85.3 µg/mg insect).<sup>88</sup> Chemicals were topically applied once to insects and insects were observed for a week. These studies suggest that a high application rate is necessary for insecticidal ability. However, in combination with plant oils (mustard, coconut, sesame or sunflower), eugenol was effective at killing grain beetles *Sitophilus granarius*, *Sitophilus zeamais*, *Tribolium castaneum* and *Prostephanus truncatus* at concentrations of 1 µL/kg grain.<sup>89</sup> The LD<sub>50</sub> of eugenol for click beetles (*Elateridae*) was 516.5 µg/larva.<sup>90</sup>

Noctuid (owlet moth) caterpillars (*Trichoplusia ni* and *Pseudaletia unipuncta*) do not appear to be sensitive to clove oil. The EC<sub>50</sub> values for reduced growth in caterpillars were 400 ppm and 690 ppm, for the two insects respectively. The LC<sub>50</sub> is 63,000 and 54,000 ppm for direct sprays of *T. ni* and *P. unipuncta* respectively, and 3,700 and 4,900 ppm for feeding.<sup>91</sup> Clove oil was administered twice to larvae at 24 hours and 168 hours post-emergence. Feeding trials were performed in Petri dishes with contaminated leaves as the food source.

Eugenol is not particularly effective against lice (*Pediculus capitis*). The LD<sub>50</sub> was 0.25 mg/cm<sup>2</sup> (or 25 kg/ha) for females and 1.0 mg/cm<sup>2</sup> for eggs.<sup>92</sup>

Eugenol and mixtures of eugenol with alpha-terpineol and cinnamic alcohol can be effective insecticides against American cockroaches (*Periplaneta americana*), carpenter ants (*Camponotus pennsylvanicus*), and German cockroaches (*Blattella germanica*). LC<sub>50</sub> values were 0.047 mg/cm<sup>2</sup> (or 4.7 kg/ha) for American cockroaches, 0.012 mg/cm<sup>2</sup> for carpenter ants, and 0.021 mg/cm<sup>2</sup> for German cockroaches.<sup>93</sup> Exposed American cockroaches demonstrated hyperactivity followed by hyperextension of the legs and abdomen, then fast immobilization followed by death. The above application rates are fairly low compared to other eugenol studies on cockroaches. One study found that 0.206 mg/cm<sup>2</sup> of eugenol rendered 50% of tested American cockroaches immobile, and 0.148 mg/cm<sup>2</sup> killed 50% of test organisms.<sup>94</sup> A follow-up study found that eugenol disrupts cell binding of octopamine in American cockroaches and fruit flies (*Drosophila melongaster*).<sup>95a, b</sup>

Eugenol is used as an acaricide against dust mites—*Dermatophagoides* spp.<sup>96</sup> and *Tyrophagus* spp.<sup>97</sup> The LD<sub>50</sub> of eugenol for *Dermatophagoides farinae* adults was 4.8 µg/cm<sup>2</sup> (0.48 kg/ha). For *Dermatophagoides pteronyssinus* adults, the LD<sub>50</sub> was 3.7 µg/cm<sup>2</sup> (0.37 kg/ha). For adult mites of the species *Tyrophagus putrescentiae*, the LD<sub>50</sub> value for eugenol is 12 µg/cm<sup>2</sup> (1.2 kg/ha).

Eugenol is an effective termiticide. Termites (*Coptotermes formosanus*) were deterred from tunneling through eugenol-treated sand barriers for at least five days. Eugenol was not a feeding deterrent when applied directly to blocks of wood.<sup>98</sup> Another study found that clove bud oil killed 100% of termites (*C. formosanus*) in 2 days at 50 µg/cm<sup>2</sup> (2 kg/ha).<sup>99</sup> In fumigation experiments, clove oil produced 100% mortality in Japanese termites at 0.5 µL/L of air.<sup>100</sup>

A number of studies evaluated the effectiveness of clove oil as a mosquito repellent and insecticide. The data suggest that clove oil is particularly toxic to mosquitoes. Eugenol kills mosquito larvae (specifically *Ochlerotatus caspius*) with an LC<sub>50</sub> values of 7.53 mg/L for 24 hours and 5.57 mg/L for 48 hours.<sup>101</sup> In *Aedes aegypti*, the LC<sub>50</sub> was 33 mg/L (unspecified exposure duration).<sup>101</sup> However, another study found higher mortality rates at marginally higher doses. Eugenol induced 100 percent mortality in mosquitos *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* at a dose of 7 L/ha in 30-35 minutes.<sup>102</sup>

**Levels of concern for insects:** There were no honeybee studies available with a specific toxicological endpoint. The LD<sub>50</sub> of 0.48 kg/ha for the mite *Dermatophagoides* is used instead. This value was divided by six to adjust the value for the fact that a NOAEL is not available. The TRV used in the MMWD assessment is 0.08 kg/ha.

### 6.3.2.C Terrestrial Plants

Clove oil causes electrolyte leakage by disrupting the waxy cuticle on the leaves of the plant, eventually causing plant death.<sup>103</sup> High application rates (approximately 10 kg/ha) are typically needed to achieve herbicidal activity.

Solutions of 1, 5 and 10% clove oil (in addition to 0.2% adjuvant and water) were applied to plants at a rate of 100 mL/m<sup>2</sup>, corresponding to an application rate of 10, 50 and 100 kg/ha.<sup>104</sup> In these studies, clove oil caused visible damage to common ragweed (*Ambrosia artemisiifolia*), common lambsquarters (*Chenopodium album*), and Johnsongrass (*Sorghum halepense*) using a 5% solution (see Figure 5 in reference 104). Curves fit to the measured doses suggest that application rates between 10-30 kg/ha would be sufficient to kill 50% of the study plants.

Leaf injury can be measured by electrolyte leakage, where electrolyte leakage is quantified by the conductivity of a leaf submerged in deionized water. At application rates of 20 kg/ha (of a 2% solution) 80% electrolyte leakage was observed.<sup>104</sup>

Another study evaluated the herbicidal activity of clove oil and eugenol on broccoli (*Brassica napa*), lambsquarters (*C. album*) and redroot pigweed (*Amaranthus retroflexus*). At concentrations equivalent to 7.5 kg/ha for eugenol and 12.5 kg/ha for clove oil, considerable electrolyte leakage occurred. Nettle (*Urtica urens*) experienced 90% foliar damage at application rates of 12-61 L/ha, whereas 21-38 L/ha were required for 90% foliar damage to purslane (*Portulaca oleracea*).<sup>103</sup>

There were no studies on the effects of clove oil on seed germination. It is not expected that clove oil will injure seeds since the method of action of clove oil is to cause electrolyte leakage from the leaves of a growing plant. However, some essential oils (clove oil was not tested) have proven effective in inhibiting seed germination in wheat.<sup>105</sup>

**Levels of concern for terrestrial plants:** The lowest clove oil concentration at which 50% of foliar plant damage was observed was 10 kg/ha for Johnsongrass and burning nettle. Because seedling emergence will likely not be affected by clove oil or eugenol, no TRV was selected for this effect.

### 6.3.2.D Microbes

Clove oil and eugenol are toxic to fungi and bacteria. Products containing clove oil have been used as antifungal, antibacterial and antiviral treatments for many years.<sup>6</sup>

Various types of fungi are sensitive to clove oil. Although the details of the studies are not available, pest fungi *Aspergillus parasiticus* and *Fusarium moniliforme*<sup>106</sup> and pecan scab fungus, or *Fusicladium effusum*<sup>107</sup> were reported to be controlled with clove oil. Eugenol inhibited *Candida albicans* and *Cryptococcus neoformans* with minimal inhibitory concentrations (MIC) of 0.625 and 0.293 mg/L respectively.<sup>108</sup> The eugenol-containing oil of the tropical plant *Croton argyrophyloides*, also inhibited *Candida albicans* at concentrations of 9-19 mg/L.<sup>109</sup>

In a study designed to look at the mechanism of eugenol toxicity to bacteria, the authors found that eugenol structurally alters the cell wall of *Escherichia coli*, *Staphylococcus aureus*,

*Salmonella enterica*, *Pseudomonas fluorescens*, and *Brochothris thermosphacta*.<sup>110</sup> Although details are unavailable, one study reports that eugenol is lethal to nematode *Meloidogyne incognita*.<sup>111</sup>

**Levels of concern for microbes:** Risks were not evaluated for microbes due to the absence of quantitative data on which to base a TRV.

### 6.3.3 Aquatic Organisms

Clove oil is frequently used at low doses as an anesthetic to fish. Higher doses can cause mortality. Clove oil toxicity to fish varies substantially between species. Exposure duration also affects toxicity, with longer exposures causing death instead of sedation. There are fewer studies for aquatic invertebrates, and considerable variation exists within the studies. There is no clove oil or eugenol toxicity information available for amphibians. Toxicity reference values derived from the available aquatic toxicity studies are highly uncertain given the paucity of data.

#### 6.3.3.A Fish

Clove oil is used as an anesthesia for fish. Efficient anesthetization is needed when fish are handled, transported or stunned. Numerous studies discuss clove oil doses which anesthetize fish and minimize handling-related stress without causing undue side effects. The studies suggest that there is an abrupt time-related transition between exposures that cause effective anesthesia and exposures which cause mortality (i.e. a dose which causes anesthesia at 15 minutes may cause death after more than one hour). However, this effect is highly species-dependent. Table G-4 in Appendix G summarizes the available data.

For a variety of saltwater fishes, a small dose of clove oil is an effective anesthesia that does not cause significant accidental death. At 40 ppt clove oil in seawater, total anesthesia is observed after 3-6 minutes for Mediterranean yellowtail *Seriola dumerili*, sea bass *Dicentrarchus labrax*, gilthead seabream *Sparus aurata*, dentex *Dentex dentex*, and sharp-snouted seabream *Diplodus puntazzo*. Total recovery occurred after 2-5 minutes with no adverse effects observed.<sup>112</sup>

The freshwater fish tambaqui was tolerant to much larger doses of eugenol. Exposure to 65 mg/L eugenol was adequate to induce a surgical anesthetic state for tambaqui (*Colossoma macropomum*), and recovery time was similar for dosages up to 100 mg/L. Exposure to 65 mg/L for up to 30 min did not cause fish mortality.<sup>113</sup> There was no mortality in tambaqui at doses of 135 mg/L (exposure duration was not reported).

Not all fish species are as tolerant as tambaqui, and some studies indicate that concentrations <70 mg/L eugenol can cause mortality in salmonids. One study found 100% mortality in rainbow trout and cherry salmon at exposures of 63 mg/L eugenol for 20 minutes. For exposure durations of 10 minutes, survival was 90% in rainbow trout, but still 0% in cherry salmon.<sup>114</sup> The study found that interspecies differences could be substantial; goldfish (*Carassius auratus*) suffered no mortality after 120 minutes at 63 mg/L. Another study found roughly similar LC<sub>50</sub> values for clove oil: The LC<sub>50</sub> for rainbow trout was 81.1 mg/L for 10 minutes and 14.1 mg/L for 96 hours.<sup>115</sup> Another study on rainbow trout found LC<sub>50</sub> values between 9 and 65 mg/L clove oil for exposure durations of 96 hours and 30 minutes, respectively.<sup>116</sup> A third study noted arrested breathing in Atlantic salmon at 100 mg/L of clove oil after 6 minutes.<sup>117</sup> The study also found that clove oil doses above 20 mg/L reduced stress related with anesthesia. Acute toxicity values

of clove oil for carp were 74.3 mg/L for a 10-minute LC<sub>50</sub> and 18.10 mg/L for a 96-hour LC<sub>50</sub>.<sup>118</sup> Acute toxicity values of clove oil for European catfish were 76.70 mg/L for a 10-minute LC<sub>50</sub> and 18.40 mg/L for a 96-hour LC<sub>50</sub>.<sup>119</sup>

Clove oil anesthesia was found to have sub-acute effects. The blood profile (erythrocyte count and volume, haemoglobin concentration, haematocrit, erythrocyte haemoglobin and leukocyte count) of rainbow trout was unaffected after anesthesia with 30 mg/L clove oil for 10 minutes.<sup>115</sup> Blood tests were taken directly following exposure and also 24 hours after exposure. A significant increase in the concentration of glucose and ammonia, and a significant decrease in aspartate aminotransferase activity were found.<sup>115</sup> No histopathological changes were demonstrated in the liver, spleen, cranial and caudal kidneys.<sup>115</sup> Another detailed pharmacokinetic study showed that eugenol has a 12 hour half-life in rainbow trout tissue and may accumulate after repeated administration.<sup>120</sup> The study used 75 mg/L for 10 minutes to anesthetize fish after a preliminary study found that 50 mg/L provided only brief (approximately 5 minute) anesthesia; 100 mg/L caused some deaths.

In summary, clove oil can serve as an anesthesia for a variety of fish; however, lengthy exposures can cause mortality and sub-acute morbidity to a variety of fish species. Figure 6-3 demonstrates the dependence of LC<sub>50</sub> on the exposure duration for Atlantic salmon. Regardless of the acute toxicity of clove oil, the anesthetic effects are likely to decrease fish survival in the wild.

**Figure 6-3:** LC<sub>50</sub> values for Atlantic salmon as a function of exposure duration. Each point represents the mean value for ten fish. *Data source:* Reference 116.

**Levels of concern for fish:** For the MMWD risk assessment, the LC<sub>50</sub> of 9 mg/L for rainbow trout was selected as both the acute and the chronic toxicity endpoint. This value was divided by 20 so the dose more closely approximated a NOEC. A factor of 20 was used instead of a factor of six because a study done on salmon suggests that adverse effects on olfaction occurred at

pesticide concentrations that were 20 times lower than the LC<sub>50</sub>.<sup>45</sup> The acute and chronic TRV for fish used in the MMWD risk assessment is 0.45 mg/L.

### 6.3.3.B Amphibians

No amphibian studies specifically evaluating toxic effects were encountered for clove oil or eugenol. However, one study was found that investigated the physiological effects of eugenol on frogs. The study describes eugenol's ability to stimulate muscle contraction in *Rana catesbeiana* by inducing Ca<sup>+2</sup> release.<sup>121</sup> This was not a traditional toxicity study and no LC<sub>50</sub> or NOELs were determined.

**Levels of concern for amphibians:** No amphibian risk assessments were performed because there were no quantitative data on which to base a TRV.

### 6.3.3.C Aquatic Invertebrates

Clove oil has also been used to anesthetize shellfish and octopuses for transport and handling. Similar to the observations for fish, the length of the exposure period can be important in distinguishing between effective, but not lethal, anesthesia. Clove oil toxicity to aquatic pathogens was slight to moderate. A summary of clove oil toxicity to aquatic invertebrates can be found in Appendix G, Table G-5.

Shellfish sensitivity to clove oil varies by species. Pearl oysters, or *Pinctada albina*, can be anesthetized with 1.5 mL/L of clove oil.<sup>122</sup> However, some species are much more sensitive. Using clove oil at concentrations of 0.125 mL/L is considered a humane way to kill the Australian giant crab *Pseudocarcinus gigas*.<sup>123</sup>

Dose and time duration are both important for effective anesthesia in octopus and prawn. Clove oil's ability to anesthetize the octopus *Octopus minor* was tested at concentrations of 50, 100, 150, 200, 250 and 300 mg/L.<sup>124</sup> There was no mortality at any of these concentrations. A concentration of 200 mg/L clove oil was found to be optimum: rapid anesthetic (4-5-minute) and recovery (12-23 minute) times were observed in the common octopus. Higher water temperatures induced quicker anesthesia and recovery. Acute toxicity and anesthetic effects of clove oil were studied in the tiger prawn *Penaeus semisulcatus*.<sup>125</sup> The 1-hour LC<sub>50</sub> and 24-hour LC<sub>50</sub>s were determined to be 130 and 30 mg/L, respectively. Clove oil can also be used as an anesthetic to the giant freshwater prawn, *Macrobrachium rosenbergii*.<sup>126</sup> At clove oil concentrations of 300 mg/L, prawns were anesthetized, but no mortality was observed.

Although eugenol can kill the garden snail, it was not a particularly effective molluscicide to the freshwater snail *Biomphalaria alexandrina*.<sup>127</sup> The LC<sub>50</sub> for eugenol in the schistosomiasis-carrying aquatic snail *Biomphalaria alexandrina* was 28 mg/L, for *Bulinus truncatus* the LC<sub>50</sub> was 24 mg/L and for *Lymnaea natalensis* it was 22 mg/L.<sup>128</sup> The chemical exposure period was

**Table 6-7: Summary of Clove Oil and Eugenol Ecotoxicity Data**

Taxa	Endpoint	Number of Studies <sup>a</sup>	Min	Dose Median	Max
Insects (mg/kg)	LD <sub>50</sub>	5	0.37	1.2 kg/ha	25
	LD <sub>50</sub>	3	5,560 <sup>a</sup>	30,000 mg/kg	31,000
	LC <sub>50</sub>	5	5.6	33 ppm	4,900
Microbes (mg/L)	MIC <sup>b</sup>	2	293 mg/L	<sup>c</sup>	625 mg/L
Fish (mg/L)	10 min–4 day	8	9	42 <sup>c</sup>	81
	LC <sub>50</sub> <sup>d</sup>				
Aquatic Invertebrates (mg/L)	1-24 hr LC <sub>50</sub>	6	22	29 <sup>f</sup>	300

<sup>a</sup> This particular value was 517 µg/insect larvae. Assuming the larvae weighed the same as a bee (0.000093 kg), the LD<sub>50</sub> becomes 5,560 mg/kg.

<sup>b</sup> MIC = Minimum inhibitory concentration, the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

<sup>c</sup> No median is reported because there were only two studies.

<sup>d</sup> Different study durations (as short as 10 minutes and as long as 4 days) are grouped. See Appendix H for the specifics. Clove oil and eugenol toxicity are particularly exposure dependent (see Figure 6-3). In the table above, the 9 mg/L dose is for a 12-hour exposure and the 81 mg/L dose is for 10 minutes of exposure to eugenol and clove oil, respectively.

<sup>e</sup> Averaged from two LC<sub>50</sub> values: 18 mg/L (96-hour, clove oil) and 65 mg/L (30 minutes, eugenol).

<sup>f</sup> Averaged from two LC<sub>50</sub> values: 30 mg/L (24-hour, clove oil) and 28 mg/L (unreported exposure duration, eugenol).

**Table 6-8: Clove Oil/Eugenol Toxicity Reference Values Used in MMWD Risk Assessments**

		MMWD			
Taxa	Exposure Type	Selected Endpoint	Dose	Adjustments to Dose	TRV Used in MMWD Risk Assessment
<b>Humans</b>					
	acute RfD	NOAEL (rat)	250 mg/kg	÷100 <sup>a</sup>	2.5
	chronic RfD	NOAEL (rat)	250 mg/kg	÷100 <sup>a</sup>	2.5
<b>Mammals</b>					
	acute	NOAEL (rat)	250 mg/kg	None	250 mg/kg
	chronic	NOAEL (rat)	250 mg/kg	None	250 mg/kg
<b>Insects</b>					
	honeybees	LC <sub>50</sub> ( <i>Dermatophagoides</i> )	0.48 kg/ha	÷6 <sup>b</sup>	0.08 kg/ha
<b>Fish</b>					
	acute	LC <sub>50</sub>	9 mg/L	÷20 <sup>b</sup>	0.45 mg/L
	chronic	LC <sub>50</sub>	9 mg/L	÷20 <sup>b</sup>	0.45 mg/L
<b>Aquatic Invertebrates</b>					
	acute	LC <sub>50</sub>	130 mg/L	÷6 <sup>b</sup>	22 mg/L
	chronic	LC <sub>50</sub>	130 mg/L	÷6 <sup>b</sup>	22 mg/L

USFS and EPA have not completed a risk assessment for clove oil. There are no TRVs to compare to the values in this Table.

<sup>a</sup> The animal NOAEL was divided by an interspecies uncertainty factor of 10 and an intraspecies factor of 10, equivalent to dividing by 100.

<sup>b</sup> The factor of six or 20 is used when there is only an LD<sub>50</sub> or LC<sub>50</sub> value available, not a NOAEL or NOEC, because the endpoint of killing 50% of the organisms is not acceptable in most cases. The factor of six is used by the US EPA in evaluation of endangered species effects and is based on a review of literature studies in which both LD<sub>50</sub> or LC<sub>50</sub> and NOAEL or NOEC values were available for comparison. The factor of 20 is used for especially sensitive species such as salmonids. See Section 6.3 for more discussion of this concept.

not discussed. The authors report a significant reduction in total trematode larvae production per snail treated with eugenol.

**Levels of Concern:** For the MMWD risk assessment, the 1-hour LC<sub>50</sub> of 130 mg/L for the tiger prawn was divided by a factor of six to obtain a TRV for aquatic invertebrates of 22 mg/L.

#### 6.3.3.D Aquatic Plants

No clove oil or eugenol studies were found for aquatic plants.

#### 6.3.4 Data Gaps

The available data suggest that clove oil is likely to be minimally toxic to mammals, but highly toxic to some insects and microbes, as well as fish and aquatic invertebrates. Given the variability in the insect data, a meta-analysis of insect studies for a variety of species would be helpful in determining patterns in clove oil's toxicity to insects, with a focus on pollinators. There are no data on the toxicity of clove oil or eugenol to amphibians. In general, conclusions for acute toxicity are drawn from a very few studies; even fewer studies exist for sub-chronic and chronic effects.

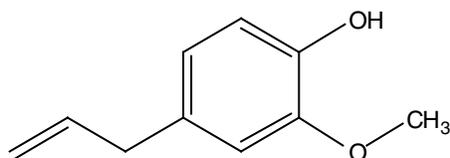
Fish and aquatic invertebrates are clearly affected by clove oil, and the FDA has expressed some concerns about using clove oil as a fish anesthetic because of potential toxicity to humans eating fish treated with clove oil.<sup>7</sup> An assessment of the bioaccumulation potential for eugenol is needed to clarify this issue.

The Matran product contains 50% eugenol and 50% of a mixture of wintergreen oil, butyl lactate and lecithin, all listed by EPA as minimal risk compounds. The toxicity of this mixture of chemicals is unknown; however runoff potential is very low under the proposed conditions of use because of rapid degradation of these compounds in the environment.

### 6.4 Environmental Fate of Eugenol

#### 6.4.1 Overview

Eugenol (CAS number 97-53-0) is an essential oil herbicide with empirical formula of C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>, derived from clove oil, an extract of *Eugenia caryophyllata*, a type of myrtle. Clove oil is comprised of around 35 different compounds that have been identified, with the primary constituent being eugenol. Pure eugenol is distilled out of the clove oil mixture for use in the Matran product, thus this section on environmental fate focuses exclusively on eugenol. See the introduction to this chapter for additional information on the composition of clove oil. The chemical structure of eugenol is shown below. Table 6-9 summarizes the chemical and physical properties of eugenol.



Eugenol

Eugenol is a very weak organic acid, with pKa of 10.19.<sup>129</sup> In aqueous solution, the acid is not substantially dissociated.

#### 6.4.2 Water Solubility and Soil Binding of Eugenol

Eugenol is a volatile liquid at room temperature and has moderate water solubility (2,463 mg/L at 25°C).<sup>129</sup> The octanol-water partition coefficient,  $K_{ow}$ , for eugenol is 186,<sup>129</sup> indicating low solubility in water relative to organic solvents and low to moderate potential for bioaccumulation, with an estimated bioconcentration factor of 31.<sup>129</sup>

The organic-carbon-adjusted soil adsorption coefficient ( $K_{oc}$ ) of eugenol is 409 mL/g,<sup>129</sup> a value that indicates that, in a mix of soil and water, eugenol is distributed in both media, with a slight preference for binding to soil. As a result, eugenol has moderate mobility in soils, adsorbing to soils and sediments to some extent.

**Table 6-9: Chemical and Physical Properties of Eugenol**

Property	Eugenol
CAS number	97-53-0
EPA PC code	102701
Molecular weight (g/mol)	164.20
Water solubility (mg/L at ~25°)	2,460
Half-life (days)	
Hydrolysis	--
Anaerobic	--
Aerobic	--
Atmospheric	
Field dissipation (soil)	--
Field dissipation (water)	--
Vapor pressure (mm Hg at ~25°C)	0.0226
$K_{oc}$ (mL/g)	409
$K_{ow}$ (20°C)	186
$K_H$ (atm·m <sup>3</sup> /mol at ~25°C)	2.0 x 10 <sup>-6</sup>

*Data source:* Reference 129.

NA = not available.

#### 6.4.3 Persistence of Eugenol

Eugenol is anticipated to be short-lived in the environment, and is rapidly dissipated and degraded via volatilization and atmospheric decomposition. Soil and water biodegradation data are not available.<sup>129</sup> Photolysis is not anticipated to be a major degradation pathway.<sup>129</sup> Eugenol does not hydrolyze readily in water, but will volatilize from water over time if microbial degradation or adsorption to sediments does not occur. No half-lives are available for eugenol in soil or water; however data on the half-life of the related compound methyl eugenol is available.<sup>130</sup> The study found that methyl eugenol has a dissipation half-life of 4-4.5 days from cigarette filters placed in or on top of the soil. Shorter half-lives were found for methyl eugenol in soil (0.33-0.5 days) and water (0.5-1.5 days).

Eugenol can be degraded or transported away from an application site through a number of different processes. The most important known processes for dissipation of eugenol are volatilization and degradation by reaction with ozone and hydroxyl radicals in the atmosphere, and adsorption to soils and sediments.

#### **6.4.3.A Microbial Degradation**

Eugenol is degraded by microbes, although specific data for microbial degradation in soils is not available. One laboratory study found that *Pseudomonas fluorescens* bacteria degraded eugenol; the responsible enzyme was isolated and characterized.<sup>131</sup> This same reaction has been used commercially to transform eugenol into the flavor molecule vanillin.<sup>132</sup> Transformation is relatively rapid, with the process being used commercially to produce vanillin. Since *Pseudomonas sp.* is a common soil bacterium, it is anticipated that microbial degradation of eugenol will be relatively rapid in soils.

#### **6.4.3.B Transport by Air**

Air transport of eugenol away from the application site can occur through spray drift during and for a short time after an application. Spray drift can contaminate soil and surface waters, damage non-target plants, and expose humans and wildlife through inhalation and dermal exposure. Post-application volatilization drift is also a significant source of off-site transport for eugenol because of its high vapor pressure (0.0226 mm Hg at 25°C).<sup>129</sup> When dissolved in water, eugenol volatilizes slowly to the air, as dictated by its low Henry's law constant of  $2.0 \times 10^{-6}$  atm-m<sup>3</sup>/mol.<sup>129</sup> The modeled volatilization half-life from a river was calculated to be 24 days; volatilization from a lake was estimated to occur with a 177-day half-life.<sup>129</sup> Volatilization is also expected to occur from wet soils, although the rate of microbial degradation may be competitive. Vapor phase eugenol reacts rapidly with hydroxyl radicals and ozone in the atmosphere, with estimated half-lives of six hours and 23 hours (dependent on ozone concentration), respectively.<sup>129</sup>

#### **6.4.3.C Transport by Water**

Eugenol volatilizes rapidly and is anticipated to be degraded rapidly in soils by microbial activity, thus it is not considered to be a potential groundwater contaminant, nor is substantial surface water runoff anticipated, except through preferential flow pathways such as cracks and crevices or gravelly soils, if rain were to occur soon after an application.

#### **6.4.3.D Uptake by Plants**

Plants treated with eugenol do not translocate the chemical through foliage or roots of the plant. There is no information available on the persistence of eugenol residues in dead plant tissue.

#### **6.4.3.E Field Studies on the Environmental Fate of Eugenol**

No field studies are available on the environmental fate and transport of eugenol.

### **6.4.4 Matran Product Profile**

The Matran product has been selected as one of the herbicides to be considered by MMWD for possible use in its Vegetation Management Plan (VMP). Matran is certified by the Organic Materials Review Institute (OMRI) for use on organic crops. It contains eugenol from clove leaf oil as the active ingredient (a.i.) at 50% weight percent. The remaining 50% of the product is comprised of wintergreen oil, butyl lactate, and lecithin.<sup>2</sup> All of these additional ingredients are listed as minimal risk inert by EPA.<sup>8</sup> The product contains 4.0 lbs/gal of eugenol. When applied as a foliar spray, Matran is mixed at 5–8% by volume in aqueous solution. Probable application rates that may be used by MMWD are anticipated to be 4–10 lbs/acre.

EPA has given this product an acute hazard warning label of “Caution”, placing it in Category 3 or 4. This rating means that the product is considered to be “Slightly toxic.” Exposure to skin or eyes may cause mild eye or skin irritation, dizziness, headache or nausea.<sup>133</sup>

### **6.5 Exposure Assessment and Risk Characterization for Eugenol**

Assessment of risk requires knowledge of both the inherent toxicity of a chemical and the amount of exposure that is anticipated based on intended uses. Risk characterization combines the hazard and exposure data to provide a picture of risks associated with herbicide use.

This exposure analysis is divided into four categories: workers, the general public, terrestrial wildlife, and aquatic life. Only foliar applications are modeled. The modeled foliar application rate of clove oil is 4.0, 8.0 and 10.0 pounds per acre for Lower, Central and Upper estimates. The Central exposure estimate provides the most likely exposure scenario and the Upper estimate represents a low-probability, worst-case event. More information about the types of exposure scenarios considered in this risk assessment is available in section 2.4. Toxicity reference values and dietary reference values for clove oil used in the analysis are discussed in Section 6.2 (humans) and Section 6.2.2 (animals and other organisms).

The USFS has not yet created an exposure worksheet for eugenol; thus, a new spreadsheet was developed by PRI for the MMWD analysis. All chemical-specific parameters are from the available literature. Peak runoff was not modeled because there are no available empirical nor modeled water contamination rates. Long-term runoff was not modeled because eugenol dissipates rapidly in the environment and is not expected to persist into the rainy season. No chronic exposures were evaluated for the same reason: clove oil has a very short dissipation half life (1–7 days) and is not expected to persist in the environment. Substantial bioconcentration in fish is not anticipated.

As with the other herbicides evaluated for the MMWD project, several additional exposure scenarios were evaluated that were not in the SERA/USFS worksheets, including drinking water exposure for birds and large mammals and all exposures for a large carnivore. Because eugenol is quite volatile, a worker inhalation exposure worksheet was also added. For water contamination, scenarios for accidental spills of concentrated and diluted clove oil product to a small, thermally stratified pond and Bon Tempe reservoir were evaluated.

An additional worksheet was developed to sum the dermal and ingestion exposures for wildlife to give aggregate doses. Aggregate worker exposures from multiple exposure events were also estimated. No aggregate exposures were estimated for the general public because of the low probability of multiple exposures.

Exposure scenarios were categorized qualitatively as “**Highly Probable**,” “**Probable**,” “**Possible**,” “**Improbable**” and “**Highly Improbable**.” These five categories are used throughout the exposure estimates to designate the likelihood of each scenario occurring. Common scenarios and their probabilities are summarized in Tables 2-8 through 2-11, starting on page 2-28. Assigned probabilities are based on the assumption that the application guidelines are followed.

For all of the different exposure scenarios, **Lower**, **Central** and **Upper** estimates were calculated. Upper exposure estimates were calculated by changing all parameters to values that increase estimates; Lower estimates were obtained by changing all parameters to values that decrease estimates; and Central estimates used parameter values that are perceived as most realistic. See Section 2.4 for a complete description of parameter values used in the calculations.

Exposure estimates for the humans and wildlife are presented and compared to toxicity reference values (TRVs) to give hazard quotients (HQs) that provide an estimate of risk for different exposure scenarios. Hazard quotients above one indicate that exposure exceeds the level of concern, and humans or wildlife may be at risk of adverse effects. Scenarios with  $HQ > 1.0$  are flagged as potentially problematic and recommendations are made for how to avoid them. Hazard quotients between 0.1 and 1.0 suggest that there may be particularly sensitive individuals or species that may be affected. Hazard quotients below 0.1 indicate low levels of risk for the effects that have been studied and are represented by the TRVs.

No risk assessments were performed for the other ingredients in the Matran product—wintergreen oil, butyl lactate, and lecithin—or the product mixture. All of the other ingredients are classified by EPA as minimal risk other ingredients. Addition of a surfactant to application mixtures is not suggested on the Matran label, and exposures to added surfactants would not be anticipated from use of Matran.

### 6.5.1 Chemical-Specific Exposure Parameters for Eugenol

Many of the parameters used to estimate exposure are constant from chemical to chemical, e.g., typical amounts of food consumed, surface area of a child and body weight, among others. These parameters and the values used in the exposure models are discussed in Section 2.4. Other parameters, such as absorption coefficients and water contamination rates, are chemical-specific and are based on physical properties such as water solubility,  $K_{ow}$ , vapor pressure,  $K_{oc}$  and half-life, as well as experimental data.

Table 6-10 presents the eugenol-specific parameters used in the calculations, including dermal absorption rates, half-lives, and air concentration estimates. As discussed in Section 2.4.3, USFS/SERA developed an estimate of dermal absorption rates and dermal permeability based on  $K_{ow}$  and molecular weight. This calculation was also used to estimate dermal absorption for glyphosate, triclopyr and clopyralid. For these chemicals, experimental data on dermal absorption rates were available for comparison to the estimated absorption rates. Although there are no dermal absorption data available for eugenol, the calculation utilizing the  $K_{ow}$  and the molecular weight is the best available estimate and was used in the risk assessment.

Estimates of airborne concentrations of eugenol at the application site due to volatilization were calculated using data from the California Air Resources Board (ARB) and Department of Pesticide Regulation (DPR) monitoring reports of pesticide applications.<sup>134</sup> The calculation used to estimate inhalation exposure is based on the vapor pressure of eugenol and is described in detail in Section 2.4.3.A.

**Table 6-10: Clove Oil-Specific Exposure Parameters**

Parameter	Lower Value	Central Value	Upper Value
First-order dermal absorption rate (h <sup>-1</sup> )	0.0037	0.011	0.035
Dermal permeability (cm/hr)	0.0044	0.0066	0.0098
Concentration in air at application site (mg/m <sup>3</sup> )	0.00264	0.0264	0.264

*Data source:* References 130 and 134.

Brenton VMS listed the following techniques as potential strategies for MMWD for controlling invasive species with clove oil:

- High volume foliar applications to control broom seedlings at 4.36-8.72 pounds per acre
- Spot foliar applications to control thistle at 4.36 pounds per acre
- Foliar applications to control annual grasses at 4.36 pounds per acre

The application rates and volumes listed in Table 6-11 were used to calculate Lower, Central and Upper exposure estimates for workers, the general public, and terrestrial and aquatic wildlife.

The foliar scenarios provide an estimate of exposures from high-volume (30–60 gallons per acre) applications. The anticipated application rates of clove oil would be: 4–10 lbs a.i./acre. A range of volumes from 31 to 67 gallons per acre is modeled, resulting in concentrations of 3–8% clove oil (by volume). Exposures from cut-stump applications are not calculated because clove oil will not be used for cut-stump applications. However, workers may transport concentrated material to the site before foliar applications. Therefore, exposures from the worst-case scenario of an accidental spill of undiluted Matran (4 lb a.i./gallon) were estimated for workers, the general public, terrestrial wildlife, and aquatic wildlife.

**Table 6-11: Application Rate and Application Volume Model Inputs for Matran**

Scenario	Parameter	Lower	Central	Upper
High-volume foliar	Application rate (lb a.i./acre)	4	8	10
	Percent a.i. (volume %)	3	3	8
	Application volume (gallons)	33	67	31

### 6.5.2 Application Methods for Clove Oil (Eugenol)

Because Matran is a burndown, non-systemic herbicide, application methods that may be used on MMWD lands for clove oil are limited to directed foliar sprays or ground sprays of cotyledons and seedlings not more than 12 inches in height. In directed foliar applications, the herbicide sprayer or container is carried by backpack and the herbicide is applied to selected target vegetation. Chemical contact with the arms, hands, or face is Highly Improbable because of the low height of the vegetation treated. To reduce the likelihood of significant exposure to legs, application crews should not walk through treated vegetation. Usually, a worker treats approximately 0.5 acre/hour with a plausible range of 0.25–1.0 acre/hour.

Ground sprays are usually conducted with a truck or tractor-mounted boom that applies the herbicide to a swath the width of the boom. Ground spray application methods typically treat approximately 16 acres/hour with a plausible range of 11–21 acres/hour.

### 6.5.3 Water Contamination Estimates

Concentration estimates for eight accidental spill scenarios were calculated. The eight spill scenarios included three spill volumes (one, 20 and 200 gallons) for a spill of the diluted product to a thermally stratified small pond and Bon Tempe reservoir. The 200 gallon spill of diluted product was added specifically for clove oil/eugenol because work crews may transport more diluted product because of the higher application rate used for clove oil/eugenol compared to the three conventional herbicides. Two additional 20-gallon spill scenarios were calculated for water contamination from a spill of concentrated product to a small pond and Bon Tempe reservoir. The spills of concentrated product are designed to represent a spill that might occur on-site during mixing, since Matran is not applied in concentrated form. All of these scenarios are considered Highly Improbable. See Section 2.4.2 for a detailed discussion of these scenarios. Results are shown in Table 6-12.

Throughout this document, the word “contaminated” is used to mean that any amount of a chemical residue is present. “Contaminated” does not necessarily equate to hazardous, but indicates only that the compound is present at some level.

Exposures from peak runoff and long-term runoff scenarios were not calculated because of the short dissipation half-life of clove oil (less than a few days) as a result of the high vapor pressure of clove oil. Clove oil quickly volatilizes and is carried away from the application site with prevailing winds. Thus, it is unlikely that significant quantities of clove oil would remain at an application site long enough to be washed away in the fall/winter rainy season. The microbial degradation half-life of clove oil is not available, but is also anticipated to be quite rapid. Therefore, only concentrations from accidental spill scenarios were modeled.

**Table 6-12: Calculated Eugenol Concentrations for Water Contamination Scenarios**

Scenario		Concentrations (mg/L)		
		Central	Lower	Upper
<b>Thermally-stratified pond</b>				
Accidental spill of diluted product	1 gal	0.21	0.23	0.59
	20 gal	4.2	4.5	12
	200 gal	42	45	120
Accidental spill of concentrated product	20 gal	145	<sup>a</sup>	<sup>a</sup>
<b>Well-mixed reservoir</b>				
Accidental spill of diluted product	1 gal	0.000011	0.000012	0.000030
	20 gal	0.00021	0.00023	0.00060
	200 gal	0.0021	0.0023	0.0060
Accidental spill of concentrated product	20 gal	0.0074	<sup>a</sup>	<sup>a</sup>

<sup>a</sup> Only a single, worst-case estimate of concentration was calculated for spills of concentrated Matran product.

For the three conventional herbicides, an additional calculation was developed to determine the maximum volume of herbicide that could be used in the MMWD watershed without exceeding herbicide concentrations that produce an HQ > 0.1, 0.5 and 1.0 for a child drinking water from the reservoir, assuming 5% and 100% long-term runoff of applied herbicide. This calculation was not done for clove oil, since since no long-term runoff is anticipated.

#### 6.5.4 Risks to Humans

Exposure estimates were performed for both workers and members of the general public. Accidental/incidental and general handling exposures were considered for herbicide applicators for ground spray, and backpack foliar applications. No exposures for clove oil cut-stump applications were calculated because this type of application is not proposed for the MMWD project. Exposure estimates for the general public were developed for the scenarios of people contacting contaminated vegetation on or near an application site, eating contaminated fruit, or drinking contaminated water. Only acute exposure scenarios were evaluated to obtain a range of exposure estimates for both worst-case and more probable scenarios.

##### 6.5.4.A Workers

Risks from accidental and general exposure scenarios were calculated for workers. Accidental exposures include wearing contaminated gloves for one minute and one hour, direct spray onto hands, and direct spray to lower legs. General exposures for backpack spraying and ground spraying were also calculated. Results are shown in Table 6-13.

The highest Central worker exposure estimate was 17 times the RfD for the Improbable event of wearing gloves contaminated by concentrated product for one hour. The high-probability exposures from general handling of the chemical in backpack spraying and ground spraying had HQs equal to 4.2% and 7.2% of the RfD, respectively.

Exposure estimates from the scenarios that are the most likely to occur for workers are highlighted below:

1. **General exposure due to backpack spraying (Highly Probable).** The Central dose estimate for general backpack spraying is 4.2% of the RfD. The Upper estimate is 32% of the RfD.
2. **General exposure due to ground spraying (Highly Probable).** The Central dose estimate for general ground spraying is 7.2% of the RfD. The Upper estimate is 60% of the RfD.
3. **Inhalation from general exposure (Highly Probable).** The Central dose estimate for inhalation due to general exposure is 0.14% of the RfD. The Upper estimate is 4.1% of the RfD.
4. **Wearing contaminated gloves for one minute (Probable).** The Upper dose estimate for wearing gloves contaminated by diluted product for one minute is 3.5% of the RfD. For the concentrated product, the dose estimate is 43% of the RfD. The Central estimates are lower: 0.83% and 28% of the RfD for diluted and concentrated products respectively.
5. **Wearing contaminated gloves without washing for one hour (Improbable).** The Upper dose estimate for wearing gloves contaminated with diluted product for one hour is 2.1 times the RfD. For concentrated product the same scenario yields a dose that is 26 times the RfD. The Central estimates are lower: 50% and 17 times the RfD for diluted and concentrated product, respectively.
6. **Accidental spill to the hands that is left unwashed for one hour (Improbable).** The Central dose estimate for a spill of diluted product on workers' hands and leaving it for one hour is 0.68% of the RfD. The Upper estimate is 6% of the RfD. The Central dose estimate

for a spill of concentrated product on workers' hands and leaving it for one hour is 23% of the RfD. The Upper estimate is 74% of the RfD.

7. **Accidental spill to the lower legs that is left unwashed for one hour (Improbable).** The Central dose estimate for a spill of diluted product to workers' lower legs for one hour is 1.7% of the RfD. The Upper estimate is 15% of the RfD. The Central dose estimate for a spill of concentrated product on workers' lower legs and leaving it for one hour is 57% of the RfD. The Upper estimate is 1.8 times the RfD.

If accidental worker exposures occur, the dose from that scenario must be added to the general exposure to obtain an aggregate dose. For example, if a worker sprays vegetation with a backpack sprayer for seven hours, inhales the chemical and also wears a glove contaminated with diluted product for one hour, the combined Central exposure estimate is  $0.11 + 0.0036 + 1.2 = 1.31$  mg/kg-day. In this case, wearing contaminated gloves for one hour yields an exposure that is 11 times the general exposure estimate and 300 times larger than the inhalation exposure. For this chemical, it is important to avoid the accidental scenarios since they are the highest exposures on a mg/kg-day basis. Confidence in these assessments is low, as dermal absorption and general exposure data for clove oil are not available.

These exposure estimates do not include splashes into the eyes, as there are no quantitative, systemic exposure estimates for this scenario. Clove oil is a mild eye irritant,<sup>2</sup> but little systemic absorption would be expected from such an event.

**Table 6-13: Estimated Eugenol Exposures and Hazard Quotients for Workers**

Scenario	Calculated Dose (mg/kg)			RfD (mg/kg-day)	Hazard Quotient (HQ)		
	Central	Lower	Upper		Central	Lower	Upper
<b>Foliar Worker Accidental/Incidental Exposures (dose in mg/kg-event)</b>							
Contaminated gloves, 1 min	0.021	0.015	0.087	2.5	0.0083	0.0059	0.035
Contaminated gloves, 1 h	1.2	0.88	5.2	2.5	<b>0.50</b>	<b>0.35</b>	<b>2.1</b>
Spill on hands, 1 h	0.017	0.0057	0.15	2.5	0.0068	0.0023	0.060
Spill on lower legs, 1 h	0.042	0.014	0.37	2.5	0.017	0.0056	<b>0.15</b>
<b>Worker Accidental/Incidental Exposures with Concentrated (no dilution) Product (dose in mg/kg-event)</b>							
Contaminated gloves, 1 min	0.71	0.47	1.1	2.5	0.28	0.19	0.43
Contaminated gloves, 1 h	43	28	64	2.5	<b>17</b>	<b>11</b>	<b>26</b>
Spill on hands, 1 h	0.58	0.18	1.8	2.5	0.23	0.073	0.74
Spill on lower legs, 1 h	1.4	0.45	4.5	2.5	<b>0.57</b>	0.18	<b>1.8</b>
<b>Foliar Worker General Exposures (mg/kg-day)</b>							
General exposure, backpack spraying	0.11	0.0018	0.80	2.5	0.042	0.00072	0.32
General exposure, ground spraying	0.18	0.0026	1.51	2.5	0.072	0.0011	<b>0.60</b>
Inhalation	0.0036	0.00012	0.10	2.5	0.0014	0.000050	0.041

RfD = Reference dose. Hazard Quotients greater than 0.1 are shaded. Hazard Quotients greater than one are **bolded**.

#### 6.5.4.B General Public

Acute clove oil exposure scenarios for the general public were evaluated for direct spray onto a person, contact with contaminated vegetation, and consumption of contaminated fruit and water. No runoff or fish consumption scenarios were evaluated because clove oil will not persist until the rainy season and it does not bioaccumulate significantly. No chronic scenarios were evaluated because clove oil has a very short half life (7 days) and is not expected to persist in the

environment. Exposure estimates for the general public are lower than for workers. The only Central exposure estimate exceeding the RfD was for the Highly Improbable scenario of a child drinking contaminated pond water after an accidental spill of concentrated product. The results are summarized in Table 6-14 below.

No exposure scenarios are considered to be Probable or Possible for pelargonic acid. It is still useful to consider the scenarios that yield the highest exposures, regardless of their probability, to evaluate the potential need for additional precautions to protect the public. For clove oil, two scenarios result in Central HQs above 0.1:

1. **Direct spray of a child over its entire body (Highly Improbable).** The Upper and Central dose estimates for a child sprayed with clove oil over its entire body are 2.3 times and 26% of the RfD, respectively.
2. **A child drinking from a thermally stratified, small pond (Highly Improbable).** The Upper dose estimates for a 20-gallon spill of diluted and concentrated clove oil to a pond are 53% and 6.4 times the RfD, respectively.
3. **A woman consuming contaminated berries (Improbable).** The Upper and Central acute dose estimates for a woman eating berries that had been sprayed with clove oil are 14% and 0.10% of the RfD. No chronic exposures were calculated.
4. **A woman brushing against contaminated vegetation (Improbable).** The Upper dose estimate for a woman brushing against contaminated vegetation is 32% of the RfD. The Central estimate was 11%.

The scenario of eating contaminated berries is Improbable if the application guidelines are followed. In order to reduce the probability of exposures, the public should be made aware of application timing and locations, and berry bushes or other edible plants should be trimmed or mowed before herbicide treatments. Conducting applications during the week instead of on the weekend, limiting access to application sites, and avoiding off-target direct sprays to blackberry, blueberry (huckleberry), thimbleberry, hazelnut, and manzanita plants will help ensure public safety.

The likelihood of exposures from brushing against contaminated vegetation can be reduced to Improbable by trimming or mowing vegetation prior to treatment.

**Water Consumption Scenarios:** Only the Highly Improbable scenario in which a child drinks from a thermally stratified pond contaminated with concentrated product resulted in Central HQs greater than 1.0 (20-gallon spill, HQ=4.4 times the RfD). A spill of 200 gallons of diluted Matran product results in a smaller Central HQ of 1.3 times the RfD.

Concentrations of clove oil from spills into a reservoir like Bon Tempe were lower than those for spills into a small pond by a factor of 20,000, and HQs are substantially less than one—0.033% of the RfD for the Upper exposure estimate for child drinking out of Bon Tempe reservoir after a 20-gallon spill of concentrated product. Adherence to the MMWD application guidelines would make a high-volume spill of concentrated product into a reservoir Highly Improbable, and with a plan in place to notify water treatment plants if such a spill were to occur, we conclude that it is

**Table 6-14: Estimated Eugenol Exposures and Hazard Quotients for the General Public**

Scenario	Receptor	Calculated Dose (mg/kg-event)			RfD (mg/kg-day)	Hazard Quotient (HQ)		
		Central	Lower	Upper		Central	Lower	Upper
<b>Acute exposure estimates for diluted clove oil product (foliar treatment)</b>								
Direct spray of child, whole body	Child	0.64	0.22	5.7	2.5	0.26	0.086	<b>2.3</b>
Direct spray of woman, feet, lower legs	Adult female	0.064	0.022	0.57	2.5	0.026	0.0087	0.23
Vegetation contact, shorts and T-shirt	Adult female	0.27	0.043	0.81	2.5	0.11	0.017	0.32
Contaminated fruit consumption	Adult female	0.094	0.013	1.9	2.5	0.038	0.0054	0.75
Water consumption (pond) after 1 gal spill	Child	0.016	0.010	0.067	2.5	0.0064	0.0042	0.027
20 gal spill	Child	0.32	0.21	1.33	2.5	0.13	0.083	0.53
Concentrated 20 gal spill	Child	11	6.7	16	2.5	<b>4.4</b>	<b>2.7</b>	<b>6.4</b>
Diluted 200 gal spill	Child	3.2	2.1	13	2.5	<b>1.3</b>	0.83	<b>5.3</b>
Water consumption (reservoir) after 1 gal spill	Child	$8.1 \times 10^{-7}$	$5.3 \times 10^{-7}$	$3.4 \times 10^{-6}$	2.5	$3.2 \times 10^{-7}$	$2.1 \times 10^{-7}$	$1.4 \times 10^{-6}$
20 gal spill	Child	0.000016	0.000011	0.000068	2.5	$6.5 \times 10^{-6}$	$4.4 \times 10^{-6}$	0.000027
Concentrated 20 gal spill	Child	0.00055	0.00034	0.00083	2.5	0.00022	0.00014	0.00033
Diluted 200 gal spill	Child	0.00016	0.00011	0.00067	2.5	0.000065	0.000042	0.00027

RfD = Reference Dose. Hazard Quotients above 0.1 are shaded. Hazard quotients greater than one are **bolded**.

Highly Improbable that drinking water quality in MMWD reservoirs will be compromised by spills of clove oil into the reservoirs.

Contamination by long-term runoff will not occur with clove oil because it dissipates rapidly in the environment and is not expected to persist into the rainy season.

### 6.5.5 Risks to Wildlife

The wildlife risk assessment is divided into two parts, terrestrial and aquatic. Exposure estimates for terrestrial wildlife are high for dermal exposure due to clove oil's high dermal permeability coefficient. Hazard quotients for aquatic wildlife were high because aquatic species are quite sensitive to clove oil and TRVs are low. At lower doses or short exposure times, clove oil is a strong anesthesia for fish and aquatic invertebrates, decreasing species' fitness for survival. At higher doses or longer exposure times, clove oil can kill sensitive aquatic species. The aquatic wildlife scenarios evaluated below are either Improbable or Highly Improbable. Long-term runoff is also unlikely given the high volatility and short half-life of clove oil acid that indicate that most of the chemical will degrade or volatilize before a runoff event.

For terrestrial wildlife, Upper exposure estimates for Possible exposures exceeded TRVs for all exposures except carnivores eating contaminated prey and small mammals eating fruit. Central exposure estimates produced hazard quotients that were mostly between 0.1 and 1. The comparatively high exposure estimates are largely a result of the high application rates used for clove oil.

For aquatic scenarios, all Upper exposure estimates exceeded the TRV for fish exposed to an acute spill in a pond. Central exposures for 20-gallon spills also exceeded the TRVs for fish. Only the 20-gallon spill of concentrated product into a pond exceeded the TRV for aquatic invertebrates.

#### 6.5.5.A Terrestrial Wildlife

Only a subset of the wildlife scenarios described in Chapter 2 were evaluated for clove oil. Tables 6-15 and 6-16 provide the acute and aggregate exposures and hazard quotients for terrestrial wildlife. Chronic exposures are not anticipated for clove oil, since it dissipates rapidly through volatilization. See Section 2.4.5 for a discussion of the methods used to estimate wildlife exposures and 6.2 for a summary of eugenol and clove oil toxicity studies and TRVs.

With the exception of drinking water after an accidental spill, all wildlife exposures were considered Possible or Probable. For clove oil applications proposed by MMWD, it is likely that TRVs for terrestrial wildlife will be exceeded. These exposure estimates may be an overestimate for herbivores because the invasive vegetation being sprayed is typically not the preferred diet of native species. Results are shown in Table 6-15.

1. **A large mammal eating contaminated vegetation, acute (Possible).** The Central acute exposure estimates for grass-eating herbivores is 55% the TRV. The Upper estimate is 1.9 times the TRV.

2. **A large bird eating contaminated vegetation, acute (Possible).** The Central acute exposure estimate for an herbivorous bird is 86% of the TRV. The Upper estimate is 3.0 times the TRV.
3. **A small mammal eating contaminated insects (Probable).** The Central acute dose estimate for a small mammal eating contaminated insects is 74% the TRV. The Upper dose estimate 2.8 times the TRV.
4. **A small bird eating contaminated insects (Probable).** The Central dose estimate for a small bird eating contaminated insects is 1.2 times the TRV. The Upper estimate is 4.5times the TRV.
5. **Consumption of contaminated prey by carnivorous mammals or birds, acute (Possible).** All estimates of exposure for all carnivorous mammals and birds are less than 13% of the TRVs.

Of the Improbable scenarios, all estimates for accidental spray of insects and small mammals resulted in HQs between 0.1 and one.

The TRVs for animals are considerably higher than human RfDs. If uncertainty factors were applied to the TRVs used for wildlife as they are for humans, hazard quotients would be higher. However, the Upper estimate uses a modeled application rate that exceeds the intended application rate, therefore Upper estimates should be viewed as Improbable worst-case scenarios.

Aggregate exposure estimates are the sum of dermal and food exposures. Water consumption was not included in aggregate exposure estimates because the only Probable water contamination scenario was long-term runoff. Runoff is anticipated to occur at least several months after the day of a direct spray or consumption of contaminated insects or vegetation. Since runoff and direct spray or eating contaminated prey do not occur within the same time window, it is impossible for wildlife to be exposed to both. USFS/SERA did not calculate aggregate exposures; we added this calculation for insectivorous and herbivorous small mammals because of their vulnerability to direct sprays and eating contaminated food in a single day. The results are presented in Table 6-16. The highest aggregate exposure to clove oil produced a hazard quotient equal to 3.4 times the TRV for the Upper exposure estimate for insectivorous small mammals. The dose contribution from contaminated food was almost five times greater than the dose from direct spray.

#### **6.5.5.B Terrestrial Plants**

For terrestrial plants, unintended direct spray will result in an exposure equivalent to the application rate. Most plants that are sprayed directly with clove oil at or near the recommended range of application rates will be damaged. Buffer zones of 25 feet are probably sufficient to protect most plants because high application rates are necessary to achieve clove oil's herbicidal effects. Less than 2% of the chemical is expected to drift more than 25 feet. Clove oil affects only plant foliage and will not have any residual herbicidal effects at the site.

**Table 6-15: Estimated Acute Eugenol Exposures and Hazard Quotients for Terrestrial Wildlife**

Scenario	Receptor	mg/kg-day or mg/kg/event			TRV (mg/kg)	Hazard Quotient (HQ)		
		Central	Lower	Upper		Central	Lower	Upper
<b>Direct Spray</b>								
First-order absorption	Small mammal	51	8.8	150	250	0.20	0.035	0.60
100% absorption of direct spray to 50% of body	Small mammal	190	97	240	250	0.78	0.39	0.97
100% absorption of direct spray to 50% of body	Honeybee	1300	640	1600	5,000	0.26	0.13	0.32
<b>Consumption of contaminated fruit and vegetation</b>								
Fruit	Small mammal	10	1.4	27	250	0.040	0.0057	0.11
Grass	Large mammal	138	69	490	250	0.55	0.28	<b>1.9</b>
Grass	Large bird	215	108	760	250	0.86	0.43	<b>3.0</b>
<b>Consumption of contaminated water</b>								
20 gal spill of diluted product into pond	Small mammal	0.62	0.66	1.7	250	0.0025	0.0026	0.0069
	Large mammal	0.27	0.29	0.76	250	0.0011	0.0012	0.0031
	Small bird	1.14	1.2	3.2	250	0.0046	0.0048	0.013
	Large bird	0.16	0.17	0.44	250	0.00064	0.00068	0.0018
20 gal spill of diluted product into reservoir	Small mammal	0.000031	0.000034	0.000088	250	1.3x10 <sup>-7</sup>	1.4x10 <sup>-7</sup>	3.5x10 <sup>-7</sup>
	Large mammal	0.000014	0.000015	0.000039	250	5.6x10 <sup>-8</sup>	6.0x10 <sup>-8</sup>	1.6x10 <sup>-7</sup>
	Small bird	0.000058	0.000062	0.00016	250	2.3x10 <sup>-7</sup>	2.5x10 <sup>-7</sup>	6.5x10 <sup>-7</sup>
	Large bird	8.02x10 <sup>-6</sup>	8.60x10 <sup>-6</sup>	0.000022	250	3.2x10 <sup>-8</sup>	3.4x10 <sup>-8</sup>	8.9x10 <sup>-8</sup>
20 gal spill of concentrated product into pond	Small mammal	21	a	a	250	0.084	a	a
	Large mammal	9.4	a	a	250	0.038	a	a
	Small bird	39	a	a	250	<b>0.16</b>	a	a
	Large bird	5.4	a	a	250	0.022	a	a
20 gal spill concentrated product into reservoir	Small mammal	0.0011	a	a	250	4.4x10 <sup>-6</sup>	a	a
	Large mammal	0.00048	a	a	250	1.9x10 <sup>-6</sup>	a	a
	Small bird	0.0020	a	a	250	8.0x10 <sup>-6</sup>	a	a
	Large bird	0.00027	a	a	250	1.1x10 <sup>-6</sup>	a	a
200 gal spill of diluted product into pond	Small mammal	6.2	6.6	17	250	0.025	0.026	0.069
	Large mammal	2.7	2.9	7.6	250	0.011	0.012	0.031
	Small bird	11	12	32	250	0.046	0.048	0.13
	Large bird	1.6	1.7	4.4	250	0.0064	0.0068	0.018
200 gal spill of diluted product into reservoir	Small mammal	0.00031	0.00034	0.00088	250	1.3x10 <sup>-6</sup>	1.4x10 <sup>-6</sup>	3.5x10 <sup>-6</sup>
	Large mammal	0.00014	0.00015	0.00039	250	5.6x10 <sup>-7</sup>	6.0x10 <sup>-7</sup>	1.6x10 <sup>-6</sup>
	Small bird	0.00058	0.00062	0.0016	250	2.3x10 <sup>-6</sup>	2.5x10 <sup>-6</sup>	6.5x10 <sup>-6</sup>
	Large bird	0.000080	0.000086	0.00022	250	3.2x10 <sup>-7</sup>	3.4x10 <sup>-7</sup>	8.9x10 <sup>-7</sup>

**Table 6-15 (cont): Estimated Acute Eugenol Exposures and Hazard Quotients for Terrestrial Wildlife**

Scenario	Receptor	mg/kg-day or mg/kg/event			TRV (mg/kg)	Hazard Quotient (HQ)		
		Central	Lower	Upper		Central	Lower	Upper
<b>Consumption of contaminated insects</b>								
	Small mammal	185	93	690	250	0.74	0.37	<b>2.8</b>
	Small bird	300	150	1100	250	<b>1.2</b>	0.60	<b>4.5</b>
<b>Consumption of contaminated small mammals</b>								
	Carnivorous small mammal	17	8.4	21	250	0.068	0.034	0.084
	Carnivorous large mammal	9.0	4.5	11	250	0.036	0.018	0.044
	Carnivorous bird	26	13	32	250	<b>0.10</b>	0.052	<b>0.13</b>

TRV = Toxicity Reference Value. Hazard Quotients above 0.1 are shaded. Hazard Quotients greater than one are **bolded**.

<sup>a</sup> Only a single, worst-case estimate of concentration was calculated for spills of concentrated Matran product.

**Table 6-16: Eugenol Aggregate Exposures and Hazard Quotients for Terrestrial Wildlife**

Animal	Scenario	Exposure Estimates (mg/kg)		
		Central	Lower	Upper
<b>Herbivorous small mammal eating fruit (TRV = 250 mg/kg)</b>				
	Direct spray, first-order absorption	51	8.8	151
	Eating fruit	10	1.4	27
	<i>Sum</i>	61	10	178
	<i>HQ</i>	<b>0.24</b>	0.041	0.71
<b>Insectivorous small mammal (TRV = 250 mg/kg)</b>				
	Direct spray, first-order absorption	51	8.8	151
	Eating insects	185	93	693
	<i>Sum</i>	236	102	844
	<i>HQ</i>	<b>0.94</b>	0.41	<b>3.4</b>

TRV = Toxicity Reference Value. Hazard Quotients above 0.1 are shaded. Hazard Quotients greater than one are **bolded**. The values that are being summed are from Table 6-15.

### 6.5.5.C Aquatic Wildlife

The calculated water concentrations of clove oil for aquatic life are the same as those used in the human and terrestrial exposure estimates for drinking water (see Table 6-10). Exposure estimates are compared to TRVs for fish and aquatic invertebrates, the only taxa for which toxicity information was available. No runoff scenarios were evaluated because of the short half-life of clove oil at the application site. Results are shown in Table 6-17.

Hazard quotients for fish exceeded one for all of the Upper exposure estimates for the pond scenarios. Only the Central and Lower one-gallon spill scenarios did not result in exposures greater than the fish TRV for pond scenarios. The aquatic invertebrate TRV is approximately 50 times greater than that for fish. Both the 20-gallon spill of concentrated product and the 200-gallon spill of diluted product into the pond exceeded the TRV for aquatic invertebrates. All scenarios for 20-gallon spills to ponds produced hazard quotients greater than 0.1. All scenarios for 20-gallon spills to Bon Tempe produced hazard quotients less than 0.1.

**Table 6-17: Estimated Eugenol Hazard Quotients for Aquatic Wildlife**

Receptor	Scenario	Hazard Quotients			TRV (mg/L)
		Central	Lower	Upper	
<b>Fish</b>					
Spill of diluted product into pond:	1 gal	0.47	0.51	<b>1.3</b>	0.45
	20 gal	<b>9.3</b>	<b>10</b>	<b>26</b>	0.45
	200 gal	<b>93</b>	<b>100</b>	<b>260</b>	0.45
Spill of concentrated product into pond:	20 gal	<b>322</b>	<sup>a</sup>	<sup>a</sup>	0.45
Spill of diluted product into reservoir:	1 gal	0.000024	0.000027	0.000067	0.45
	20 gal	0.00047	0.00051	0.0013	0.45
	200 gal	0.0047	0.0051	0.013	0.45
Spill of concentrated product into reservoir:	20 gal	0.016	<sup>a</sup>	<sup>a</sup>	0.45
<b>Aquatic Invertebrates</b>					
Spill of diluted product into pond	1 gal	0.0095	0.010	0.027	22
	20 gal	0.19	0.20	0.54	22
	200 gal	<b>1.9</b>	<b>2</b>	<b>5.4</b>	22
Spill of concentrated product into pond	20 gal	<b>6.6</b>	<sup>a</sup>	<sup>a</sup>	22
Spill of diluted product into reservoir	1 gal	$5.0 \times 10^{-7}$	$5.5 \times 10^{-7}$	$1.4 \times 10^{-6}$	22
	20 gal	$9.6 \times 10^{-6}$	0.000011	0.000027	22
	200 gal	0.000096	0.00011	0.00027	22
Spill of concentrated product into reservoir	20 gal	0.00034	<sup>a</sup>	<sup>a</sup>	22

TRV = Toxicity Reference Value. Hazard Quotients greater than 0.1 are shaded. Hazard Quotients greater than one are bolded.

## References for Chapter 6

- <sup>1</sup> Schmid R. 1972. A resolution of the *Eugenia-Syzygium* controversy (Myrtaceae). *Amer J Bot* 59(4): 423–436.
- <sup>2</sup> EcoSmart. Matran label.
- <sup>3</sup> US EPA. 2004. *Floral Attractants, Repellents, and Insecticides*. US Environmental Protection Agency. November 2004. [http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet\\_florals.htm](http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_florals.htm).
- <sup>4</sup> <http://www.cosmeticsdatabase.com>
- <sup>5</sup> USPTO. 1989. Eugenol enhancement of transdermal drug delivery. US Patent 4888362. <http://www.patentstorm.us/patents/4888362/description.html>.
- <sup>6</sup> Chaieb K, Hajlaoui H, Zmantar T, *et al.* 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae): A short review. *Phytotherapy Res* 21: 501–506.
- <sup>7</sup> US FDA. 2007. *Guidance for Industry: Concerns Related to the Use of Clove Oil as an Anesthetic for Fish*, Guideline No. 150. US Food and Drug Administration Center for Veterinary Medicine. April 24, 2007. <http://www.fda.gov/cvm/Guidance/guide150.htm>.
- <sup>8</sup> US EPA. 2000. Pesticide Registration (PR) Notice 2000-6. Minimum Risk Pesticides Exempted under FIFRA Section 25(b): Clarification of Issues. US Environmental Protection Agency. May 7, 2000. [http://www.epa.gov/PR\\_Notices/pr2000-6.pdf](http://www.epa.gov/PR_Notices/pr2000-6.pdf).
- <sup>9</sup> Alma MH, Ertas M, Nitz S, *et al.* 2007. Chemical composition of content of essential oil from the bud of cultivated Turkish Clove. *BioRes* 2(2): 265–269.
- <sup>10</sup> US EPA. 2006. *Methyl Eugenol (ME) (203900) Fact Sheet*. US Environmental Protection Agency. September 2006. [http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet\\_203900.htm](http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_203900.htm).
- <sup>11</sup> US NTP. 2000. Final Report on Carcinogens Background Document for Methyleugenol. US National Toxicology Program. Dec. 13–14, 2000 meeting of the NTP Board of Scientific Counselors Report on Carcinogens Subcommittee. [http://ntp-server.niehs.nih.gov/ntp/htdocs/LT\\_rpts/tr491.pdf](http://ntp-server.niehs.nih.gov/ntp/htdocs/LT_rpts/tr491.pdf).
- <sup>12</sup> Personal communication with Judy Hight of Ecosmart, manufacturer of Matran, July 23, 2008.
- <sup>13</sup> WHO. 2006. Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives, *JECFA No. 1529: Eugenol*. World Health Organization, Joint FAO/WHO Expert Committee on Food Additives. Top-level page: [http://www.inchem.org/documents/jecfa/jecval/jec\\_841.htm](http://www.inchem.org/documents/jecfa/jecval/jec_841.htm); *JECFA Monograph 530: WHO Food Additives Series 17*, <http://www.inchem.org/documents/jecfa/jecmono/v17je10.htm>.
- <sup>14</sup> WHO. 2006. Eugenol and Related Hydroxyallylbenzene Derivatives. *JECFA Monograph 530: WHO Food Additives Series 1138*. World Health Organization, Joint FAO/WHO Expert Committee on Food Additives. <http://www.inchem.org/documents/jecfa/jecmono/v56je09.pdf>
- <sup>15</sup> US NTP. 1983. *Carcinogenesis Studies of Eugenol (CAS No. 97-53-0) in F344/N Rats and B6c3F<sub>1</sub> Mice (Feed Studies)*. US National Toxicology Program Technical Report Series No. 223. December 1983. [http://ntp-server.niehs.nih.gov/ntp/htdocs/LT\\_rpts/tr223.pdf](http://ntp-server.niehs.nih.gov/ntp/htdocs/LT_rpts/tr223.pdf).
- <sup>16</sup> Jirovetz L, Buchbauer G, Stoilova I, Stoyanova A, Krastanov A, Schmidt E. 2006. Chemical composition and antioxidant properties of clove leaf essential oil. *J Agric Food Chem* 54(17): 6303–6307.
- <sup>17</sup> Wenqian G, Shufen L, Ruixiang Y, *et al.* 2007. Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and three other traditional extraction methods. *Food Chem* 101: 1558–1564.
- <sup>18</sup> Srivastava AK, Srivastava SK, Syamsundar KV. 2005. Bud and leaf essential oil composition of *Syzygium aromaticum* from India and Madagascar. *Flavour Fragr J* 20: 51–53.

- 
- <sup>19</sup> Lee K-G, Shibamoto T. 2001. Antioxidant property of aroma extract isolated from clove buds [*Syzygium aromaticum* (L.) Merr. et Perry]. *Food Chem* 74: 443–448.
- <sup>20</sup> Raina VK, Srivastava SK, Aggarwal KK *et al.* 2001. *Flavour Frag J* 16: 334–336.
- <sup>21</sup> Vernin G, Vernin E, Metzger J, *et al.* 1994, as cited in reference 20.
- <sup>22</sup> Gopalakrishanan and Narayanan. 1988, as cited in reference 20.
- <sup>23</sup> Prashar A, Locke IC, Evans, CS. 2006. Cytotoxicity of clove (*Syzygium aromaticum*) oil and its major components to human skin cells. *Cell Prolif* 39: 241–248.
- <sup>24</sup> Koch G, Magnusson B, Nobréus N, *et al.* 1973. Contact allergy to medicaments and materials used in dentistry. IV. Sensitizing effect of eugenol-colophony in surgical dressing. *Odontol Revy* 24(2): 109-114.
- <sup>25</sup> Koch G, Magnusson B, Nyquist G. 1971. Contact allergy to medicaments and materials used in dentistry. II. Sensitivity to eugenol and colophony. *Odontol Revy* 22(3): 275-289.
- <sup>26</sup> Rothenstein AS, Booman KA, Dorsky J, *et al.* 1983. Eugenol and clove leaf oil: a survey of consumer patch-test sensitization. *Food Chem Toxicol* 21(6): 727-733.
- <sup>27</sup> Trongtokit Y, Rongsriyam Y, Komalamisra N, *et al.* 2005. Comparative repellency of 38 essential oils against mosquito bites. *Phytother Res* 19(4): 303-309.
- <sup>28</sup> Hartnoll G, Moore D, Douek D. 1993. Near fatal ingestion of oil of cloves. *Arch Dis Child* 69(3): 392-393.
- <sup>29</sup> Dobroriz AM, Borisenko AN, Kliza VV. 2004. [A death case of eugenol intoxication]. *Sud Med Ekspert* 47(5): 45-46. [Russian]
- <sup>30</sup> Brown SA, Biggerstaff J, Savidge GF. 1992. Disseminated intravascular coagulation and hepatocellular necrosis due to clove oil. *Blood Coagul Fibrinolysis* 3(5): 665-668.
- <sup>31</sup> Eisen JS, Koren G, Juurlink DN, *et al.* 2004. N-acetylcysteine for the treatment of clove oil-induced fulminant hepatic failure. *J Toxicol Clin Toxicol* 42(1): 89-92.
- <sup>32</sup> Janes SE, Price CS, Thomas D. 2005. Essential oil poisoning: N-acetylcysteine for eugenol-induced hepatic failure and analysis of a national database. *Eur J Pediatr* 164(8): 520-522.
- <sup>33</sup> Lane BW, Ellenhorn MJ, Hulbert TV, *et al.* 1991. Clove oil ingestion in an infant. *Hum Exp Toxicol* 10(4): 291-294.
- <sup>34</sup> Kirsch CM, Yenokida GG, Jensen WA, *et al.* 1990. Non-cardiogenic pulmonary oedema due to the intravenous administration of clove oil. *Thorax* 45(3): 235-236.
- <sup>35</sup> Centers Disease Control. 1985. Illnesses possibly associated with smoking clove cigarettes. *MMWR* 34: 297-299.
- <sup>36</sup> Wright DA, Payne JP. 1962. A clinical study of intravenous anaesthesia with a eugenol derivative, G.29.505. *Br J Anaesth* 34: 379-385.
- <sup>37</sup> Ho YC, Huang FM, Chang YC. 2006. Mechanisms of cytotoxicity of eugenol in human osteoblastic cells in vitro. *Int Endod J* 39(5): 389-393.
- <sup>38</sup> Prashar A, Locke IC, Evans CS. 2006. Cytotoxicity of clove (*Syzygium aromaticum*) oil and its major components to human skin cells. *Cell Prolif* 39(4): 241-28.
- <sup>39</sup> Buch JG, Dikshit RK, Mansuri SM. 1988. Effect of certain volatile oils on ejaculated human spermatozoa. *Indian J Med Res* 87: 361-363.
- <sup>40</sup> Raghavenra H, Diwakr BT, Lokesh BR, *et al.* 2008. Eugenol—the active principle from cloves inhibits 5-lipoxygenase activity and leukotriene-C4 in human PMNL cells. *Prostaglandins Leukot Essent Fatty Acids* 74(1): 23-27.
- <sup>41</sup> Saeed SA, Gilani AH. 1994. Antithrombotic activity of clove oil. *J Pak Med Assoc* 44(5): 112-115.

- 
- <sup>42</sup> Someya H, Higo Y, Ohno M, et al. 2008. Clastogenic activity of seven endodontic medications used in dental practice in human dental pulp cells. *Mutat Res* 650(1):3 9-47.
- <sup>43</sup> CA DPR. *Pesticide Illness Surveillance Program*. California Department of Pesticide Regulation. <http://www.cdpr.ca.gov/docs/whs/pisp.htm>.
- <sup>44</sup> *American Association of Poison Control Centers*. <http://www.aapcc.org/dnn/NPDS/AnnualReports/tabid/125/Default.aspx>
- <sup>45</sup> US EPA. 2004. *Analysis of risks to endangered and threatened salmon and steelhead*. US Environmental Protection Agency. December 1, 2004. <http://www.epa.gov/espp/litstatus/effects/triclopyr/analysis.pdf>.
- <sup>46</sup> Tucker and Leitzke. 1979 in reference 45.
- <sup>47</sup> (a) Hasler AD, Scholz AT. 1983. *Olfactory Imprinting and Homing in Salmon*. New York: Springer-Verlag.
- (b) Moore A, Waring CP. 1996. Sublethal effects of the pesticide diazinon on the olfactory function in mature male Atlantic salmon parr. *J Fish Biol* 48:758-775.
- <sup>48</sup> WHO. Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives, *Eugenol No. 1529*, WHO Food Additives Series 17, <http://www.inchem.org/documents/jecfa/jecmono/v17je10.htm>.
- <sup>49</sup> Guénette SA, Ross A, Marier JF, et al. 2007. Pharmacokinetics of eugenol and its effects on thermal hypersensitivity in rats. *Eur J Pharmacol* 562(12): 60–67.
- <sup>50</sup> Han EH, Hwang YP, Jeong TC, et al. 2007. Eugenol inhibits 7,12-dimethylbenz[a]anthracene-induced genotoxicity in MCF7 cells: Bifunctional effects on CYP1 and NAD(P)H:quinone oxidoreductase. *FEBS Lett* 581(4): 749–756.
- <sup>51</sup> Hitchcock, 1952 in reference 48.
- <sup>52</sup> Hartiala et al., 1966 in reference 48.
- <sup>53</sup> Sober et al., 1950 in reference 48.
- <sup>54</sup> Taylor et al., 1964 in reference 48.
- <sup>55</sup> Lauber & Hollander, 1950 in reference 48.
- <sup>56</sup> Beroza et al., 1975 in reference 14.
- <sup>57</sup> Jenner et al., 1964 in reference 48.
- <sup>58</sup> Caujolle & Meynier, 1960 in reference 48.
- <sup>59</sup> Fujii et al., 1970 in reference 48.
- <sup>60</sup> Guenette SA, Beaudry F, Marier JF, et al. 2006. Pharmacokinetics and anesthetic activity of eugenol in male SpragueDawley rats. *J Vet Pharmacol Ther* 29(4): 265–270.
- <sup>61</sup> Damiani CE, Rossoni LV, Vassallo DV. 2003. Vasorelaxant effects of eugenol on rat thoracic aorta. *Vascul Pharmacol* 40(1): 59–66.
- <sup>62</sup> Lahlou S, Interaminense LF, Magalhães PJ, et al. 2004. Cardiovascular effects of eugenol, a phenolic compound present in many plant essential oils, in normotensive rats. *J Cardiovasc Pharmacol* 43(2): 250–257.
- <sup>63</sup> Feng J, Lipton JM. 1987. Eugenol: antipyretic activity in rabbits. *Neuropharmacology* 26(12): 1775–1779.
- <sup>64</sup> Trubek Laboratories Inc., 1958 in reference 14.
- <sup>65</sup> Hirose et al. 1987 in reference 14.
- <sup>66</sup> Hagan et al., 1965 in reference 48.

- 
- <sup>67</sup> Hagan et al., 1967 in reference 48.
- <sup>68</sup> NTP, 1981 in reference 48.
- <sup>69</sup> Miller *et al.* 1983 in reference 14.
- <sup>70</sup> IARC. 1985. *Summaries and Evaluations: Eugenol*. International Agency for Research on Cancer. 36: 75. <http://monographs.iarc.fr/ENG/Monographs/vol36/volume36.pdf>.
- <sup>71</sup> Van Duuren & Goldschmidt, 1976 in reference 48.
- <sup>72</sup> Hitchcock, 1952 in reference 48.
- <sup>73</sup> Green & Savage, 1978 in reference 48.
- <sup>74</sup> Swanson et al., 1979 in reference 48.
- <sup>75</sup> Hikiba H, Watanabe E, Barrett JC, et al. 2005. Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. *J Pharmacol Sci* 97(1): 146–152.
- <sup>76</sup> Maralhas A, Monteiro A, Martins C, et al. 2006. Genotoxicity and endoreduplication inducing activity of the food flavouring eugenol. *Mutagenesis* 21(3): 199–204.
- <sup>77</sup> Kim SS, Oh OJ, Min HY, et al. 2003. Eugenol suppresses cyclooxygenase2 expression in lipopolysaccharidestimulated mouse macrophage RAW264.7 cells. *Life Sci* 73(3): 337–348.
- <sup>78</sup> Munerato MC, Sinigaglia M, Reguly ML, et al. 2005. Genotoxic effects of eugenol, isoeugenol and safrole in the wing spot test of *Drosophila melanogaster*. *Mutat Res* 582(12): 87–94.
- <sup>79</sup> Miller *et al.* 1979 in reference 48.
- <sup>80</sup> Van Duuren et al., 1966 in reference 48.
- <sup>81</sup> Kabuto H, Tada M, Kohno M. 2007. Eugenol [2methoxy4(2propenyl)phenol] prevents 6hydroxydopamineinduced dopamine depression and lipid peroxidation inductivity in mouse striatum. *Biol Pharm Bull* 30(3): 423–427.
- <sup>82</sup> EC. 2000. *Towards the establishment of a priority lists of substances for further evaluation of their role in endocrine disruption: Preparation of a candidate list of substances as a basis for priority setting*. European Commission DG ENV. June 21, 2000. M0355008/1786Q. [http://ec.europa.eu/environment/docum/01262\\_en.htm#bkh](http://ec.europa.eu/environment/docum/01262_en.htm#bkh).
- <sup>83</sup> PANNA. 2008. Clopyralid. *PesticideInfo Web Site*. Pesticide Action Network North America. [http://www.pesticideinfo.org/Detail\\_Chemical.jsp?Rec\\_Id=PC36359](http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC36359).
- <sup>84</sup> West TF. 1944. Toxicity to flies of derivatives of eugenol and cis-and trans-isoeugenol. *Nature* 154: 488-488.
- <sup>85</sup> Ebert TA, Kevan PG, *et al.* 2007. Oral toxicity of essential oils and organic acids fed to honey bees (*Apis mellifera*). *Journal of Apicultural Research* 46: 220-224.
- <sup>86</sup> Ajayi FA, Lale NES. 2001. Seed coat texture, host species and time of application affect the efficacy of essential oils applied for the control of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored pulses. *International Journal of Pest Management* 47(3): 161-166.
- <sup>87</sup> Brown HA, Minott DA, *et al.* 1998. Biological activities of the extracts and constituents of pimento, *Pimenta dioica* L. against the southern cattle tick, *Boophilus microplus*. *Insect Science and its Application* 18(1): 9-16.
- <sup>88</sup> Huang Y, Ho SH, *et al.* 2002. Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *J Stored Products Res* 38(5): 403-412.
- <sup>89</sup> (a) Obeng-Ofori D, Reichmuth C. 1999. Plant oils as potentiation agents of monoterpenes for protection of stored grains against damage by stored product beetle pests. *Int J Pest Management* 45(2): 155-159.

(b) Obeng-Ofori D, Reichmuth C. 1997. Bioactivity of eugenol, a major component of essential oil of *Ocimum suave* (Wild) against four species of stored-product Coleoptera. *Int J Pest Management* 43(1): 89-94.

<sup>90</sup> Waliwitiya R, Isman MB, *et al.* 2005. Insecticidal activity of selected monoterpenoids and rosemary oil to *Agriotes obscurus* (Coleoptera: Elateridae). *Journal of Economic Entomology* 98(5): 1560-1565.

<sup>91</sup> Akhtar Y, Yeoung YR, *et al.* 2008. Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta*. *Phytochemistry Reviews* 7(1): 77-88.

<sup>92</sup> Yang YC, Lee SH, Lee WJ, *et al.* 2003. Ovicidal and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. *J Agric Food Chem* 51(17):4884-4888.

<sup>93</sup> Enan E. 2001. Insecticidal activity of essential oils: octopaminergic sites of action. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 130(3): 325-337.

<sup>94</sup> Ngoh SP, Choo LEW, *et al.* (1998). Insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach, *Periplaneta americana* (L.). *Pesticide Science* 54(3): 261-268.

<sup>95</sup> (a) Enan E. 2005. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. *Archives of Insect Biochemistry and Physiology* 59(3): 161-171.

(b) Price DN, Berry MS. 2006. Comparison of effects of octopamine and insecticidal essential oils on activity in the nerve cord, foregut, and dorsal unpaired median neurons of cockroaches. *Journal of Insect Physiology* 52(3): 309-319.

<sup>96</sup> Cho JH, Sung BK, *et al.* 2004. "Acaricidal components of medicinal plant oils against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*." *Journal of Microbiology And Biotechnology* 14(3): 631-634.

<sup>97</sup> Kim EH, Kim HK, *et al.* 2003. Acaricidal activity of clove bud oil compounds against *Tyrophagus putrescentiae* (Acari: Acaridae). *Applied Entomology and Zoology* 38(2): 261-266.

<sup>98</sup> Cornelius ML, Grace JK, *et al.* 1997. Toxicity of monoterpenoids and other natural products to the formosan subterranean termite (Isoptera: Rhinotermitidae). *Journal of Economic Entomology* 90(2): 320-325.

<sup>99</sup> Zhu BC, Henderson G, Chen F, *et al.* 2001. Evaluation of vetiver oil and seven insectactive essential oils against the Formosan subterranean termite. *J. Chem. Ecol.* 27(8):1617-1625.

<sup>100</sup> Park IK, Shin SC 2005. Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus* kolbe). *Journal of Agricultural And Food Chemistry* 53(11): 4388-4392.

<sup>101</sup> Knio KM, Usta J, *et al.* 2008. Larvicidal activity of essential oils extracted from commonly used herbs in Lebanon against the seaside mosquito, *Ochlerotatus caspius*. *Bioresource Technology* 99: 763-768.

<sup>102</sup> Bhatnagar M, Kapur KK *et al.* 1993. Laboratory evaluation of insecticidal properties of *Ocimum basilicum* Linnaeus and *O. sanctum* Linnaeus plant's essential oils and their major constituents against vector mosquito species. *Journal of Entomological Research (New Delhi)* 17(1): 21-26.

<sup>103</sup> Boyd NS, Brennan EB. 2006. Burning nettle, common purslane, and rye response to a clove oil herbicide. *Weed Technology* 20: 646-650.

<sup>104</sup> Tworkoski T. 2002. Herbicide effects of essential oils. *Weed Science* 50:425-431.

<sup>105</sup> Dudai N, Poljakoff-Mayber A, Mayer AM, Putievsky E, Lerner HR. 1999. Essential oils as allelochemicals and their potential use as bioherbicides. *J Chem Ecol* 25(5): 1079-1089.

<sup>106</sup> Juglal S, Govinden R, *et al.* 2002. Spice oils for the control of co-occurring mycotoxin-producing fungi. *Journal of Food Protection* 65(4): 683-687.

- 
- <sup>107</sup> Barnes GL. 1963. "In vitro toxicity of various fixed and essential oils to the pecan scab fungus, *Fusicladium effusum*." *Plant Dis Repr* 47((2)): 114-117.
- <sup>108</sup> Boonchird C, Flegel TW. 1982. Invitro Antifungal Activity Of Eugenol And Vanillin Against *Candida-Albicans* And *Cryptococcus-Neoformans*. *Canadian Journal of Microbiology* 28(11): 1235-1241.
- <sup>109</sup> Fontenelle ROS, Morais SM, *et al.* 2008. Antifungal activity of essential oils of *Croton* species from the Brazilian Caatinga biome. *Journal of Applied Microbiology* 104(5): 1383-1390.
- <sup>110</sup> Di Pasqua R, Betts G, Hoskins N, Edwards M, Ercolini D, Mauriello G. 2007. Membrane Toxicity of Antimicrobial Compounds from Essential Oils. *Journal of Agricultural and Food Chemistry* 55: 4863-4870.
- <sup>111</sup> Ding Q, Luo W, *et al.* 2007. "Toxicity comparison of thirteen phytochemicals to *Meloidogyne incognita*. *Journal of Plant Resources and Environment* 16(3): 35-39.
- <sup>112</sup> Garcia-Gomez, A, de la Gandara F, *et al.* 2002. Use of clove, *Syzygium aromaticum* L. (Merr. & Perry), oil as a cost-effective anesthetic for routine tasks in handling cultured marine fish. *Boletin Instituto Espanol de Oceanografia* 18(1-4): 21-23.
- <sup>113</sup> Roubach R, Gomes LC, *et al.* 2005. Eugenol as an efficacious anesthetic for tambaqui, *Colossoma macropomum* (Cuvier). *Aquaculture Research* 36(11): 1056-1061.
- <sup>114</sup> Hussein MMA, Wada S, *et al.* 2000. Antimycotic activity of eugenol against selected water molds. *Journal of Aquatic Animal Health* 12(3): 224-229.
- <sup>115</sup> Velisek J, Svobodova Z, *et al.* 2005. Effects of clove oil anaesthesia on rainbow trout (*Oncorhynchus mykiss*). *Acta Veterinaria Brno* 74(1): 139-146.
- <sup>116</sup> Keene *et al.* 1998. The efficiency of clove oil as an anesthetic for rainbow trout, *Orcorhynchus mykiss*. *Aquaculture Research* 29(2) 89-101.
- <sup>117</sup> Iverson *et al.* 2003. The efficacy of metomidate, clove oil, Aui-S and Benzoak as anesthetics in Atlantic salmon smolts and their potential stress-reducing capacity. *Aquaculture* 221: 549-566.
- <sup>118</sup> Velisek J, Svobodova Z, *et al.* 2005. Effects of clove oil anaesthesia on common carp (*Cyprinus carpio* L.). *Veterinarni Medicina* 50(6): 269-275.
- <sup>119</sup> Velisek J, Wlasow T, *et al.* 2006. Effects of clove oil anaesthesia on European catfish (*Silurus glanis* L.). *Acta Veterinaria Brno* 75(1): 99-106.
- <sup>120</sup> Guenette SA, Uhland FC *et al.* 2007. Pharmacokinetics of eugenol in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 266(1-4): 262-265.
- <sup>121</sup> Lofrano-Alves MS, Oliveira EL, Damiani CEN, Kassouf-Silva I, Fogaca RTH. 2005. Eugenol-induced contractions of saponin-skinned fibers are inhibited by heparin or by a ryanodine receptor blocker. *Can. J. Physiol. Pharmacol.* 83: 1093-1100.
- <sup>122</sup> Norton JH, Dashorst M, *et al.* 1996. An evaluation of some relaxants for use with pearl oysters. *Aquaculture* 144(1-3): 39-52.
- <sup>123</sup> Gardner C. 1997. Options for humanely immobilizing and killing crabs. *Journal of Shellfish Research* 16(1): 219-224.
- <sup>124</sup> Seol DW, Lee J, *et al.* 2007. Clove oil as an anesthetic for common octopus (*Octopus minor*, Sasaki). *Aquaculture Research* 38(1): 45-49.
- <sup>125</sup> Soltani M, Marmari G, *et al.* 2004. Acute toxicity and anesthetic effects of clove oil in *Penaeus semisulcatus* under various water quality conditions. *Aquaculture International* 12(4-5): 457-466.
- <sup>126</sup> Coyle SD, Dasgupta S, *et al.* 2005. Comparative efficacy of anesthetics for the freshwater prawn *Macrobrachium rosenbergii*. *Journal of The World Aquaculture Society* 36(3): 282-290.

- 
- <sup>127</sup> Radwan MA, El-Zemity SR. 2007. Naturally occurring compounds for control of harmful snails. *Pakistan Journal of Zoology* 39: 339-344.
- <sup>128</sup> El-Din ATS. 2006. Molluscicidal effect of three monoterpenes oils on schistosomiasis and fascioliasis vector snails in Egypt. *Journal of the Egyptian Society of Parasitology* 36(2): 599-612.
- <sup>129</sup> HSDB. 2006. National Library of Medicine, TOXNET. *Eugenol*. Hazardous Substances Data Bank. revised Feb. 2003. <http://toxnet.nlm.nih.gov>.
- <sup>130</sup> Shaver TN, Bull DL. 1980. Environmental fate of methyl eugenol. *Bull Environm Contam Toxicol* 24: 616-619.
- <sup>131</sup> Furukawa H, Nagasawa N. 1998. Novel Double Bond-Transferring Hydroxylation Reaction Involved in Microbial Metabolism of Eugenol. In Enzyme Engineering XIV, *Ann New York Acad Sci* 864: 81-86. <http://www.annalsnyas.org/cgi/content/abstract/864/1/81>.
- <sup>132</sup> WWUM. 2006. *Biotechnical Production of Vanillin*. Westfalische Wilhelms-Universitat Munster. <http://mibi1.uni-muenster.de/Biologie.IMMB.Steinbuechel/en/Forschung/vanillin.html>.
- <sup>133</sup> EcoSmart. Matran MSDS.
- <sup>134</sup> CA DPR. *Toxic Air Contaminant Program Air Monitoring Reports*. California Department of Pesticide Regulation. <http://www.cdpr.ca.gov/docs/emon/pubs/tac/tacstdys.htm>.